AD_____

MIPR NUMBER B6GTM7805

TITLE: Comparative Neurobehavioral Toxicity Assessment of Three Hydrocarbon Fuels

PRINCIPAL INVESTIGATOR: Alan F. Nordholm, Ph.D.

CONTRACTING ORGANIZATION: Naval Medical Research Institute
Wright-Patterson AFB, OH 45433

REPORT DATE: August 1998

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	August 1998	Final (25 Jul 97 - 24 Jul 98)

4. TITLE AND SUBTITLE

Comparative Neurobehavioral Toxicity Assessment of Three Hydrocarbon Fuels

5. FUNDING NUMBERS

MIPR B6GTM7805

6. AUTHOR(S)

Nordholm, Alan F., Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Naval Medical Research Institute
Wright-Patterson AFB, OH 45433

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

There is increasing evidence that repeated human occupational exposure to low concentrations of fuels can result in significant changes in neurobehavioral capacity. Recent evidence indicates that repeated exposure of rodents to jet fuel vapor/aerosol can induce significant changes in physiological function. To evaluate the neurobehavioral consequences of repeated fuel vapor exposures, groups of 32 male Sprague-Dawley rats were exposed for 6 hr/day, 5 days/week, for 6 consecutive weeks, to JP-8 (1 mg/L) jet fuel, JP-5 jet fuel, or diesel fuel vapor in whole body inhalation chambers. Three groups of 16 control rats each were similarly exposed to room air. Following whole body exposures, rats were rested for 65 days then were evaluated for neurobehavioral changes on a battery of ten performance tests selected from the Neurobehavioral Toxicity Assessment Battery (NTAB). Following sacrifice, rats were assayed for changes in brain or serum neurotransmitter levels, and for functional or anatomical changes in the lungs, liver and testes. Results of this investigation are compared to data reported previously for fuel industry workers exposed to jet fuel vapors for up to 32 years.

14. SUBJECT TERMS

Jet Fuels ... Neurobehavioral Toxicity ... Animal Models ... Rats ... JP-8 ... JP-5 ... Diesel Fuel ... Neurotransmitters ... NTAB .

15. NUMBER OF PAGES

83

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

N/A Where copyrighted material is quoted, permission has been obtained to use such material.

N/A Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

N/A Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

N/A In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

N/A For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

23 Sept 98

PI - Signature Date

COMPARATIVE NEUROBEHAVIORAL TOXICITY ASSESSMENT OF THREE HYDROCARBON FUELS

[1]A.F. NORDHOLM, [2]G.D. RITCHIE, [3]W.J. MALCOLM, [2]S. McINTURF, [1]L.J. TUBBS, [2]C.Y. ADEMUJOHN, [1]W.K. ALEXANDER, [1]C.L. WILSON, [2]G.B. BRIGGS, [3]Z. HONG [1]K.R. STILL, [1]J. ROSSI III, AND [1]R.L. CARPENTER

[1]NAVAL HEALTH RESEARCH CENTER DETACHMENT -TOXICOLOGY (NHRC-TD), [2]GEO-CENTERS, INC., AND [3]MANTECH ENVIRONMENTAL TECHNOLOGY, 2612 FIFTH STREET, BUILDING 433, WRIGHT-PATTERSON AIR FORCE BASE (WPAFB), OH 45433 USA

Address correspondence to: Dr. Glenn D. Ritchie at NHRC/TD, 2612 Fifth St., Building 433, WPAFB, OH 45433, USA. Telephone: 937-255-6058, Ext. 206; Fax: 937-656-7094; E-mail: gritchie@navy.al.wpafb.af.mil

ABSTRACT

There is increasing evidence that repeated human occupational exposure to low concentrations of fuels can result in significant changes in neurobehavioral capacity. Recent evidence indicates that repeated exposure of rodents to jet fuel vapor/aerosol can induce significant changes in physiological function. To evaluate the neurobehavioral consequences of repeated fuel vapor exposures, groups of 32 male Sprague-Dawley rats were exposed for 6 hr/day, 5 days/week, for 6 consecutive weeks, to JP-8 (1 mg/L) jet fuel, JP-5 jet fuel, or diesel fuel vapor in whole body inhalation chambers. Three groups of 16 control rats each were similarly exposed to room air. Following whole body exposures, rats were rested for 65 days then were evaluated for neurobehavioral changes on a battery of ten performance tests selected from the Neurobehavioral Toxicity Assessment Battery (NTAB). Following sacrifice, rats were assayed for changes in brain or serum neurotransmitter levels, and for functional or anatomical changes in the lungs, liver and testes. Results of this investigation are compared to data reported previously for fuel industry workers exposed to jet fuel vapors for up to 32 years.

[1]This research was funded by the U. S. Army Medical Research and Materiel Command (USAMRMC). The opinions and assertions contained herein are those of the authors, and are not to be construed as those of the U.S. Army or the military service at large. The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals", Institute of Laboratory Animal Resources, National Research Council, DHHS, Publication No. (NIH) 86-23 (1996).

INTRODUCTION

There is increasing evidence that repeated exposure to doses below Threshold Limit Values (TLV) of one or more of a diversity of chemical toxicants can result in persisting changes in neurobehavioral capacity (Burbacher, 1993). These changes can range from acute performance-reducing physiological irritancy effects to severe persisting changes in physical or mental health. The exposure effects may, as in the cases of Idiopathic Environmental Intolerance (IEI; formerly Multiple Chemical Sensitivity) or Sick Building Syndrome (SBS), be debilitative to the subject in the most severe case, or may in lesser cases remain undetected without evaluation using specialized neurobehavioral tests. The IEI syndrome may, for example, occur following a single or repeated exposure to one or more chemical toxicants, may begin from days to months post-exposure, may increase in severity over time without development of reliable medical biomarkers, may generalize to include adverse reactions to a diversity of previously neutral chemical stimuli, and may selectively impact only a small percentage of the exposed population (Bell *et al.*, 1997a, 1997b).

Repeated exposure to jet fuel vapor is a potential problem for fuel manufacturing, transporting, and handling workers, as well as for aircraft manufacturing, maintenance and operational personnel in both military and civilian sectors (Harris *et al.,* 1997). In the specific case of military aircraft fuel cell maintenance personnel, unprotected exposure to as high as 15,000 mg/L jet fuel vapor/aerosol has been reported. It is estimated that the U.S. military is currently consuming nearly 2.2 billion gallons of jet fuels per year. Jet fuels, containing potentially neurotoxic compounds including benzene, toluene, xylene, and n-hexane, as well as

numerous other short- and long-chain aromatic and aliphatic hydrocarbons (Mattie *et al.,* 1991), have recently been combined with thermal stability additives whose neurobehavioral toxicity potential may be unresearched (Wolfe *et al.,* 1997). Because half-lives for volatile organic chemical (VOC) components of jet fuel (*i.e.,* toluene and xylene) may vary from several hr to nearly two days, it is probable that repeated daily exposures to jet fuels may result in additive VOC concentrations in body tissues that exceed those expected from a single exposure.

Acute laboratory exposure to jet fuel vapor (JP-8) in humans has been reported only to be non-irritating to the eyes, slightly irritating to the skin, and to result in a weak dermal sensitizing potential (Kinkead *et al.,* 1992). However, a report was published (Porter, 1990) describing effects of the accidental exposure to JP-5 vapor of two US aviators during acrobatic flight. The pilots reported burning eyes, nausea, fatigue, impairment of eye-hand coordination, euphoria and memory deficits. Long-term toxicity testing of jet fuel vapor and aerosol (JP-8) in rats (exposure to 0-1000 mg/m3 for 90 days) indicated limited toxicity and no tumor formation (Mattie *et al.,* 1991; 1995). A developmental toxicity study of JP-8 vapor exposure indicated that the fuel is not a teratogen in the rat (Cooper and Mattie, 1996), although daily oral gavage dosing (0-3000 mg/kg/day) of male rats in this study resulted in decreased body weights, nephropathy, gastritis, perianal dermatitis and elevated liver enzymes. Dossing *et al.* (1985), studying the effect in fuel-filling attendants of repeated exposure to jet fuel, reported significantly enhanced antipyrine clearance, indicating fuel-related induction of hepatic metabolism. Pfaff *et al.* (1995), exposing rats to 520 mg/m^3 JP-8 vapor/aerosol for 1 hr/day for 7 days, reported an increase in dynamic lung compliance and pulmonary resistance, with a decrease in substance P concentrations from the bronchoalveolar lavage fluid (BALF). Finally, it has been recently shown (Harris *et al.,* 1997)

that nose-only exposure of mice for 1 hr/day for 7 days to varying concentrations (100 - 2500 mg/m^3) of vaporized/aerosolized JP-8 jet fuel resulted in profound immunotoxicity, as evidenced by reductions in splenic and thymic weights, loss of viable immune cell numbers, and a compromise in immune function.

Perhaps due to the minimal carcinogenicity, tumorigenesis and developmental toxicity risks from acute or chromic jet fuel exposure, and because documented physiological consequences have been limited to non-central nervous system tissues (Bruner *et al.,* 1993) there have been few published studies targeting neurobehavioral toxicity in humans, and virtually none using animals (Macys *et al.,* 1992). Several studies (Knave *et al.,* 1976, 1978, 1979; Struwe and Wennberg, 1983; Struwe *et al.,* 1983) have, however, reported the occurrence of neurasthenia (fatigue, anxiety, mood disorders, memory difficulties and psychosomatic symptoms), psychasthenia, polyneuropathy, changes in sensorimotor speed and attention, EEG abnormalities, and possible sexual dysfunction in civilian and military aircraft workers. These workers were typically exposed to jet fuel vapor/aerosols of 250-300 mg/m^3 in the manufacturing environment for from 4-32 years.

A relatively larger number of studies have been published investigating the neurobehavioral consequences of brief or chronic exposure of humans or animals to component chemicals of hydrocarbon jet fuels, including toluene (Benignus, 1981; Struwe and Wennberg, 1983; Odkvist *et al.*, 1987; Pryor, 1991; Forkman *et al.*, 1991; Ikeda *et al.,* 1993; von Euler *et al.,* 1993; Bushnell *et al.,* 1994; Miyagawa *et al.,* 1995; Niklasson *et al.*, 1995; Rahill *et al.,* 1996), xylene (Gralewicz *et al.*, 1995) and benzene (Contreras *et al.,* 1979; Gralewicz *et al.,* 1997). Human or animal effects from these exposures include vertigo and dizziness, headache,

body and joint pain, cardiac palpitations, nausea and gastritis, fatigue, tremor, vigilance deficits, increased reaction time, motor incoordination, euphoria, mild hallucinations, emotional hostility, memory deficits, insomnia, reduced ability to concentrate, and increased latency of responding, without reductions in accuracy on complex performance tests. In the extreme case, Contreras *et al.* (1979) reported progressive development of absence-like seizures, subclinical seizures and generalized seizures in cats exposed repeatedly to toluene or benzene, hypothesizing a kindling mechanism.

The present study will investigate the longer-term neurobehavioral consequences of repeated exposure of rats to levels of JP-8 jet fuel, JP-5 jet fuel, or diesel fuel vapor insufficient to induce observable physiological irritancy effects. Results of these investigations will be compared to completed results (Ritchie *et al.,* in press) reporting the neurobehavioral consequences of repeated exposure of Sprague-Dawley rats to JP-4 jet fuel vapor. A battery of brain and blood serum neurotransmitter analyses were conducted to identify reliable biomarkers in exposure groups in which a neurobehavioral change was observed. Additionally, lung, liver and testes tissues will be examined for evidence of exposure-induced toxicity.

METHODS AND MATERIALS

Materials

JP-8 Jet Fuel. JP-8 jet fuel stock was procured from the Fuels Laboratory, Wright-Patterson AFB, OH. The JP-8 formulation was mixed by the Fuels Laboratory for international scientific testing applications, and is intended to contain an "average" content of each of the major carbon short-chain compounds from JP-8 jet fuel stocks prepared by the major

U.S. oil refiners for sale to the U.S. military. The JP-8 sample was subjected to high resolution gas chromatograph (Perkin-Elmer 8500 GC with a flame ionization detector) analysis, and this result was compared for purity to the spectra provided by the Fuels Laboratory, Wright-Patterson AFB, OH.

JP-5 Jet Fuel. JP-5 jet fuel stock was procured from the Fuels Laboratory, Wright-Patterson AFB, OH. The JP-5 was provided in two 5-gallon sealed containers procured from two different jet fuel manufacturing companies. The two JP-5 samples were thoroughly mixed, subjected to high resolution gas chromatograph (GC) analysis, and this result was compared for purity to the spectra provided by the Fuels Laboratory, Wright-Patterson AFB, OH.

Diesel Fuel. Diesel fuel stock will be procured from the Fuels Laboratory, Wright-Patterson AFB, OH. The diesel fuel sample will be subjected to GC analysis, and this result was compared for purity to the spectra provided by the Fuels Laboratory, Wright-Patterson AFB, OH.

Animals

For each of three exposures (JP-8, JP-5 or diesel fuel), forty-eight (32 vapor-exposed + 16 control) adult Sprague-Dawley rats, [Crl:CD (BR)] (300-360 g at the time of exposure) were (or will be) procured from Charles River Breeding Laboratories, Raleigh, NC. Prior to neurobehavioral testing rats are pair housed in hanging polycarbonate shoe box cages with cellulose fiber contact bedding (Cell-Sorb Plus, A. W. Products, Inc., New Philadelphia, OH). During neurobehavioral testing procedures, rats are individually housed in similar cages. Fresh pelleted food (Purina Formulab #5002, Purina Mills, Inc., St. Louis, MO) and fresh conditioned

(reverse osmosis) water is available *ad libitum*. Room air temperature and humidity are maintained at 23° ± 2°C and 55% ± 15%, respectively. Rodent cage racks are located within Bio-Clean air mass displacement units that provided a constant supply of HEPA-filtered air. Electronically controlled full spectrum fluorescent light are provided on a 12:12 hour light:dark cycle; all exposures and testing occur during the light cycle. Before and during whole body inhalation exposures, rats are housed in an animal housing room adjacent to the laboratory containing the whole body inhalation chambers. Following the completion of exposures, the rats are housed in a similar housing facility (without Bio-Clean air mass displacement units) located adjacent to the laboratory spaces utilized for neurobehavioral testing. Throughout all experimental procedures, rats are weighed on a daily basis and are examined by a member of veterinary services staff for signs of physical debilitation.

Pre-Exposure Conditions

Upon receipt, all rats are quarantined for 14 days. For five days following quarantine each rat is handled and gentled for 10 min per day. Following handling procedures, rats are assigned randomly (n = 48) to either the fuel vapor (n = 32) or air control (n = 16) exposure conditions.

Exposure Conditions

Three groups of 48 rats (32 test and 16 control animals) are exposed to fuel (JP-8, JP-5 or diesel fuel) or air control atmospheres for 6 hr/day (800 hr-1400 hr), for 5 days/week (Mon-Fri), for six consecutive weeks (30 days; 180 hr total) in one of three 270-L Hinners-type whole body exposure chambers (Hinners *et al.*, 1968). During the exposures, animals are deprived of both

food and water. Rats in the fuel-exposed groups are assigned using a semi-randomization program to one of 32 possible housing locations within two of the chambers on each day of exposure to minimize effects from possible regional differences in fuel vapor concentration within the chamber. Similarly, rats assigned to the air control group are assigned using a semi-randomization program to one of 16 possible housing locations within the remaining chambers on each day. Each housing location consisted of a wire mesh cage (in an 8-cage housing rack) 17 cm (l) x 12 cm (w) x 17 cm (h), designed to maximize vapor flow-through. Air control rats in one chamber experience room air flowing at 72 $\pm$ 2 L/min. Vapor exposed rats in the remaining two chambers experience room air mixed with fuel vapor.

Generation, Delivery and Analysis of Fuel Vapor

JP-8 Jet Fuel. The inhalation exposure system consisted of a fuel vapor generator, three 270-L Hinners-type whole body exposure chambers, and a short-path infrared (IR) spectrometer (Miran 1A, Foxboro Analytical, Wilks Infrared Center, South Norwalk, CT) used for analytical purposes. The JP-8 fuel vapor was generated in a vapor generator, consisting of a packed J-tube glass column (heated to 87 $\pm$ 5°C) through which liquid JP-8 was passed top-down. An air stream counter-flow system, delivered through the column at a flow rate of 14 $\pm$ 2 L/min bottom-up, introduced the heated vapor into the inhalation system. The vapor was diluted and cooled by the main room air supply (25 $\pm$ 2.5°C) before it was introduced into two of the three Hinners-type inhalation chambers. Heat taping of input ducts was required to minimize vapor condensation. Vapor was delivered, using a push-pull system, at a flow rate of 72 $\pm$ 2 L/min through the top stack of each chamber, passed through the rat housing racks, and exhausted

through spider exhaust valves located in the base of the chambers. Air control rats in a third Hinners-type chamber were exposed to identical conditions, except that no vapor was added to the room air input (72 $\pm$ 2 L/min). Auditory volume within the Hinners-type chambers related to the inflow and outflow of control or test atmospheres averaged 85 $\pm$ 2 dB, and the chambers very illuminated only by the room lighting.

Although 250-300 individual peaks were detected through off-line high resolution gas chromatograph (Perkin-Elmer 8500 GC with a flame ionization detector) of the JP-8 jet fuel, only the concentrations of the seven largest peaks, representing typically the aliphatic hydrocarbons of C9 - C15, were routinely monitored. The on-line monitoring of the vapor concentrations within the two test chambers was accomplished by pumping air samples into the Miran-1A IR spectrometer equipped with a 12 cm cell. The C-H single bond stretch frequency at 3.48 microns, typical of kerosene-type hydrocarbons 2,3, was used to measure concentrations of the fuel vapor. The IR spectrometer was calibrated as required by vaporizing hexane in known quantities into a Tedlar bag. During the daily 6-hr inhalation exposures, the control background (room air) and JP-8 vapor concentration within the chambers were measured and adjusted every 30 min. The JP-8 jet fuel vapor concentration was targeted at 1.0 mg/L $\pm$ 10%, and was maintained by adjusting by altering the temperature and/or flow rate of the vapor generator, or the flow rate to the inhalation chambers. Daily measurements of relative humidity, temperature, and barometric pressure were made at the time of exposure and were excluded as significant causes of variability in JP-8 vapor concentrations. The intra-day and inter-day relative standard deviation of JP-8 vapor concentrations were 4.0% and 15.4%, respectively, during the initial 21 exposure days. The mean deviation of JP-8 vapor delivery concentration between the two

exposure chambers was approximately 11%. Laboratory room air was continuously monitored by IR spectrometer and by a MIE multipoint aerosol sensor (Model Ram-s). The chamber vapor chromatographs for the seven major peaks measured resembled closely those of the original jet fuel, with only a slight shift to the lighter fractions of JP-8 jet fuel components. The spent fuel was essentially devoid of jet fuel components lighter than C9 hydrocarbons. Approximately 50% of the input fuel was recovered as flow-by or in-line condensate, although no aerosol was ever detected when chamber atmospheres were sampled with Gelman 25-mm, extra thick glass fiber filters (Gelman Sciences, Ann Arbor, MI).

Post-Exposure Conditions

Following each daily 6-hr exposure, rats are removed from the inhalation exposure housing cages and returned to the home cages where *ad lib* food and water are available. To eliminate possible off-gassing exposure of room air control animals, both pair members within each home cage are exposed to either fuel vapor or room air control conditions. Rats exposed to fuel vapor are housed within a Bio-Clean air mass displacement unit different from the one housing the air control exposed rats. Each animal is weighed and inspected by the veterinary services staff for evidence of physiological irritancy or other medical debilitation.

Post Exposure Resting

Following the completion of inhalation exposures, all rats are rested for 65 days. During the rest periods, rats are pair housed by random assignment in the home cages with *ad lib* access to food and water, and were weighed and examined on a daily basis.

NTAB Testing

Following either the 65-day rest, all rats are evaluated on each of ten (10) neurobehavioral tests selected from the NTAB (Ritchie *et al.,* 1995). Selection of NTAB tests from the larger battery was based on the assumed capacity of individual tests to identify neurobehavioral deficits in rats similar to those observed previously in human aircraft workers chronically exposed to jet fuel vapor (Knave *et al.,* 1976, 1978, 1979; Struwe and Wennberg, 1983; Struwe *et al.,* 1983). All rats experience the identical NTAB tests in the same order of presentation (*i.e.*, the Conspecific Contact test always precedes the Porsolt Forced Swim test, *etc.*). For each individual test, the fuel-exposed and air control rats are evaluated in randomized order, with the experimenters blinded to the exposure history of individual subjects. Completion of NTAB sub-battery testing requires approximately 25 days.

Test	Abbr.	NTAB	Measures
1	CC	Conspecific Contact Test	Socialization
2	PFS	Porsolt Forced Swim Test	Depression
3	TFR	Tail Flick Response	Nociception
4	ASR/PPI	Acoustic Startle Response/Pre-Pulse Inhibition	Inhibitory Tone
5	OTPA	One-Trial Passive Avoidance	Short-Term Memory
6	FGS	Forelimb Grip Strength	Muscular Tone
7	TLA	Total Locomotor Activity	General Activity
8	ARAS	Appetitive Reinforcer Approach Sensitization	CNS Sensitization
9	MWM	Morris Water Maze	Spatial Localization
10	TPF	Treadmill Physical Fatigue	Chronic Fatigue

Description of NTAB Tests

Test 1. Conspecific Contact Test (CC). The CC test is a commonly accepted evaluation of conspecific socialization in rats. For the CC test, randomly selected pairs of air control or vapor-exposed rats are placed within the left and right chambers of a Social Contact apparatus (Panksepp *et al.*, 1995) for 15 min. The Social Contact apparatus consists of two clear plastic, double-sized hanging rat cage chambers (24" long x 12" high x 12" wide) that are adjoined at one end, with 1" diameter (d) nose-poke openings at each end. Each rat can either place its nose through the common nose-poke hole to potentially initiate contact with a conspecific, or through the remaining nose-poke hole which is open to the laboratory space. The dependent measures for the CC test are: (a) the number of times the experimental rat interrupts a photo beam in the common nose-poke hole versus the number of times the rat interrupts the photo beam in the nose-poke hole at the opposite end of the alley and (b) the duration of a possible 900 sec that the experimental rat interrupts the photo beam in the common nose-poke hole versus the nose-poke hole at the opposite end of the alley.

Test 2. Porsolt Forced Swim Test (PFS). The PFS test is a widely accepted measurement of emotional depression in rodents, which has been validated through measurement of rodents following administration of human anti-depressants. The PFS apparatus consists of three (3) clear Plexiglas 24" high tubes with 8" inner d. Each tube, located within a standard water bath tank, is filled with temperature controlled water (26°C $\pm$ 2°) to a depth of 12", such that a rat placed within the tube cannot contact the base of the tube with its feet or tail without submersion of the nares below the water surface. Rats are individually placed within one of the three tubes for up to 15 min, and allowed to swim or float within the apparatus. The dependent

measures for Day 1 performance in the PST are: (a) number of sec swimming; (b) number of complete circular revolutions within the apparatus; (c) number of "swimming" dives, wherein the nares are submerged below the water line; (d) number of sec immobile; and (e) number of sec from placement within the apparatus to observance of 10 sec of continuous floating (immobility). If the nares of a rat remain submerged for > 5 sec, the subject is removed from the apparatus and the trial terminated. On the following day, each rat is returned to the PFS apparatus for a maximum of 15 min, and the time to floating (immobility) compared to the Day 1 performance as a measure of learning and memory. Rat performance is recorded using video cameras for subsequent evaluation of dependent measures by an independent rater.

Test 3. Tail Flick Response (TFR). Rats are individually evaluated for integrity of nociceptive systems using the Tail-Flick Analgesia Meter w/250-500 g Rat Restrainer (Model 1420-D-30w/1434-2-D-30, Columbus Instruments, Columbus, OH). Each rat is placed in the a clear Plexiglas rat restrainer tube such that the protruding posterior two-thirds of the tail is across the sensing slot of the apparatus. At this point, a manual switch is activated initiating a timer and opening a shutter, exposing the tail to heat from a bulb located under the apparatus stage. In response to flicking of the tail, the timer is automatically stopped and the shutter closed. The dependent measure is latency (sec) from the opening of the shutter to activation of the photo beam, indicating removal of the tail from the heat source. Each rat is tested for 10 trials separated by an intertrial interval (ITI) of < 10 sec. The apparatus, with 25 heat intensity settings, is operated on setting 12, providing sufficient heat intensity to induce tail flicking but insufficient to induce tissue damage within 5 sec. If a subject fails to flick its tail for 5 sec, the trial is terminated and a maximum score of 5 sec assigned.

Test 4. Acoustic Startle Response/Prepulse Inhibition (ASR/PPI). The ASR test is a commonly used evaluation of brain stem integrity and general neurological function, while the PPI of ASR is a well accepted measurement of inhibitory tone. Randomly selected groups of four (4) rats each are placed within four Acoustic Startle Response System (Model SR-Lab, San Diego Instruments, San Diego, CA) restraint tubes (250-500 g rat model) housed within four standard sound and light attenuation chambers. The chambers are illuminated with 40W standard light bulbs. All boxes are routinely calibrated and equated for startle platform sensitivity and startle tone intensity. Rats are placed within the restraint tubes such that the head is oriented in the direction of an audio speaker located approximately 3 cm from the restraint tube door. Stimulus intensities listed below reflect the mean measured sound intensity reaching the subjects within the startle tube with the forward and rear doors in the closed position. Each animal receives 8 discreet auditory startle sessions (10 startles per session) during a single testing period. Sessions during which the rat receives only a series of eight 105 dB tones constitutes the Acoustic Startle Response (ASR) trials. Sessions during which rats receives a series of eight 105 dB tones preceded (100 ms) by a 74 dB tone are the Prepulse Inhibition (PPI) trials. A single session containing eight presentations of a 74 dB tone alone is used as a baseline control for the intensity of the startle response. Total length of the eight auditory startle sessions is approximately 23 min; a 60 sec adaptation period precedes testing, individual sessions are separated by 60 sec intersession intervals, and individual pulses within sessions are separated by 10 sec intertrial intervals. The dependent variable for each trial is amplitude (SDI, or San Diego Instruments units) of the startle response. The order of presentation of the 8 sessions is as follows:

Session	Abbr.	Description
Session 1	ASR-1	105 dB Single Startle Pulse (8 presentations)
Session 2	Control	74 dB Single Startle Pulse
Session 3	PPI-1	74 dB Pre-Pulse Paired with 105 dB Startle Pulse
Session 4	ASR-2	105 dB Single Startle Pulse
Session 5	PPI-2	74 dB Pre-Pulse Paired with 105 dB Startle Pulse
Session 6	ASR-3	105 dB Single Startle Pulse
Session 7	PPI-3	74 dB Pre-Pulse Paired with 105 dB Startle Pulse
Session 8	ASR-4	105 dB Single Startle Pulse

Test 5. One Trial Passive Avoidance Test (OTPA). The OTPA test is a commonly utilized evaluation of learning and memory in rodents. Each rat is individually placed within a the illuminated chamber of a Two-Chamber Shuttle Box (Reflex-16 Shuttle Box, Columbus Instruments, Columbus, OH). The shuttle box, with black Plexiglas flooring, sides and lid (except for an observation port on the lighted side), is equally divided into lighted and non-illuminated chambers separated by a Plexiglas divider, with a 8 x 10 cm door that can be manually lifted or closed. The floor of the apparatus consists of 3 mm d metal grid bars spaced at approximately 1 cm. The grid bar flooring of the non-illuminated chamber can be electrified, providing five brief negative reinforcements (approximately 1 ma for 0.5 sec) separated by 1 sec intervals. A single 40 watt (W) standard light bulb, mounted above the white, translucent ceiling of the illuminated chamber, provides the only illumination in the apparatus. The laboratory is illuminated with a 25 W red light to minimize visual cues observed by the rat through the clear Plexiglas. Rats are placed initially in the lighted chamber, with the animal facing the rear wall of the apparatus, and the door dividing the chambers is immediately opened. When the rat crosses completely (four limbs) from the illuminated chamber into the non-illuminated chamber the divider door is closed and the negative reinforcements administered. The time from placement in the illuminated chamber to crossing to the non-illuminated chamber is recorded. After a 5 sec

additional interval, the subject is removed from the apparatus and returned to the home cage for a 48-hr rest period. Following the 48-hr rest period, each rat is returned to the illuminated chamber and the time required for crossing into the non-illuminated chamber (defined as contact of the forepaws with the grid bar flooring) is measured, with a maximum latency of 10 min.

6. Forelimb Grip Strength (FGS). Hand-held rats are allowed to grasp the mesh pull screen of a Standard Grip Strength Meter for Rats (1027S-D-30; front limb testing unit only, Columbus Instruments, Columbus, OH). When a firm grip is observed, the rat is slowly pulled (held at the base of the tail) at a constant force in a linear direction parallel to the floor surface at a speed of approximately 3 cm/sec until release of both forepaws was observed. At this point, the digital output of the Grip Strength Meter (peak force in kg) is recorded, and another trial begun. Each animal is tested for ten trials. The dependent measure is the mean peak grip strength force (kg) calculated for the ten trials.

Test 7. Total Locomotor Activity (TLA). Randomly selected groups of four (4) rats each are evaluated for total locomotor activity. Rats are placed individually in one of four 17" x 17" Opto-Varimex Animal Activity Meters (Model Opto-Varimex-3, Columbus Instruments, Columbus, OH) for 30 min. The only illumination in the experimental room is provided by a 25W red bulb suspended near the activity meters. Through use of auto-track features (two 8 x 8 infrared light beam matrices for horizontal plane motion and vertical rearing) the following measures were automatically recorded:

(a) Horizontal beam interruptions (total number/30 min, as measured at 10 min intervals)
(b) Vertical beam interruptions (total number/30 min)
(c) Time in motion versus time immobile.
(d) Time exhibiting stereotypic movements.

Test 8. Appetitive Reinforcer Approach Sensitization (ARAS). The ARAS test is a measurement of CNS sensitization, and of dopamine system sensitization, specifically (Panksepp *et al.*, 1997). Individually housed rats are provided access to 7-10 g raw hamburger meat at approximately 1600 hr on each of two days separated by a 24 hr period. The amount of hamburger meat consumed is calculated at 800-900 hr of the following day by weighing any hamburger remaining in the home cage. Rats are then tested for appetitive reinforcer approach sensitization 48 hr following the second raw hamburger meat presentation. Rats are individually placed for 600 sec each within a 30 x 90 x 30 (high) cm non-illuminated rectangular chamber constructed of 1/2" clear Plexiglas sides and a 1/2" clear Plexiglas floor covered on the bottom side with black contact paper. In each of the four corner junctions of the chamber, a 15 cm wide x 30 cm high rectangular piece of white plastic, fluorescent light fixture grating (1.2 cm x 1.2 cm plastic mesh) is mounted to form, with the side walls of the apparatus, a triangular shaped stimulus display area. The removable grating barrier permits visual and olfactory recognition to the rat of the hamburger meat stimulus without tactile access. Behind the two stimulus holding areas at one end of the rectangular box are placed 10 g of raw hamburger meat on 4 cm d plastic weighing dishes set on 4 cm high cylindrical, clear Plexiglas pedestals. Within the remaining two stimulus display areas at the opposite end of the chamber are placed identical lids and pedestals containing no hamburger meat stimulus. Concentric semicircles (0.5 cm wide) with a 15 cm radius are etched into the bottom of the Plexiglas flooring, using each corner of the apparatus as the semicircle's center, to define four stimulus approach areas. These etched semicircles are used to define rat approach to the stimulus display areas containing the hamburger meat versus similar approach to the non-baited corners. A 15 cm radius circle is etched from the geometric center of

the apparatus to locate initial placement of the rats within the apparatus. The apparatus is rotated 180° after every two consecutive trials to counterbalance possible rat directional preferences, the two grating barriers defining the baited stimulus presentation areas are switched with those defining the non-baited stimulus presentation areas after every fourth consecutive trial, and the apparatus is thoroughly washed with 100% methanol between trials to minimize odor cues. To begin a trial, the rat is placed by one experimenter into the center circle facing neither the wall between the two baited stimuli nor the wall between the two non-baited stimuli. One of two independent observers records the number of sec (of a 600 sec maximum) that each individual rat spends with at least two forepaws on or across the semicircular lines (in the stimulus approach zones) etched near the two baited stimulus display areas, while the a second observer records the number of sec spent in the stimulus approach zones near the two non-baited stimulus display areas. Number of crosses between the baited and unbaited halves of the apparatus is recorded as a measure of locomotor activity. Room illumination is provided by a 25W red bulb suspended over the apparatus.

Test 9. Morris Water Maze (MWM). The MWM is a commonly utilized apparatus for evaluating deficits in spatial localization and memory. The MWM apparatus consists of a 48" d x 30" high metal stock tank (Quality Farm and Fleet, Inc., Xenia, OH), painted white on the interior, and filled with water to a depth of 24". Contained within the trough is a 22" high, 5" d white escape pedestal that is located 6" from the trough wall at a point that is diagonally 38" from the location designated as the rat entry point. The water, maintained at 26°C $\pm$ 2° through use of a submersible heating unit (Model #15003 1500W Sinking Stock Tank De-Icer, Agri-Master, Inc., Cosmos, MN) is tinted opaque white through addition of white tempura paint

(Sax Art Supplies, New Berlin, WI). The swimming performance of subjects is recorded and analyzed using a Videomex-V Animal Tracking System w/Water Maze Software (Columbus Instruments, Columbus, OH). A naive rat is placed into the water, facing the tank wall, at the designated entry point and allowed to swim until locating the surface of the submerged escape pedestal. Following a 5-sec rest on the pedestal, the rat is removed from the apparatus, dried with a towel, and placed in a 4-rat cage unit that is warmed with a heat lamp. Squads of 4 rats are trained 5 trials each on Day 1 in an alternating manner with an ITI of approximately 5-7 min. Dependent measures recorded for each trial are: animal path length, latency to target, dwell time in apparatus quadrants, initial heading, and number of entries into each apparatus quadrant. On Day 2, each rat is allowed two additional trials in the apparatus to measure recall. If a subject experiences submersion of the nares for 5 sec, the rat is removed from the apparatus and the trial terminated. The water is cleared of feces with a fish net following each trial.

Test 10. Treadmill Physical Fatigue (TPF). Rats are evaluated for exercise fatigue utilizing a Two-Lane Rat Exerciser Treadmill Unit (Model 0931-D-30, Columbus Instruments, Columbus, OH). The Two-Lane Treadmill Unit features troughs at the end of each running lane allowing delivery of negative reinforcement (physical contact with ice bags). Animals are initially pretrained on the apparatus for five min each at a forward lane speed of 0.1 m/sec with an inclined lane angle of 15°, a speed and inclination angle at which all tested animals easily avoid negative reinforcement. During the pretraining period each animal experiences several negative reinforcements related to entry into the trough. Twenty-four hr following the pretraining, randomly selected groups of two rats each are placed on the two lanes of the treadmill and allowed to walk for up to 45 min (2700 sec) at a lane speed of 0.250 m/sec, with a

lane inclination of 15°. The dependent measure of physical fatigue is latency to the fifth contact of the hind quarters of the subject with the negative reinforcer.

Subject Sacrifice and Biomarker Testing

Within 12-24 hr of the completion of NTAB testing, rats will be individually sacrificed in randomized order by CO_2 overdose. At each necropsy, 2-5 ml blood samples will taken from the vena cava for subsequent analysis. The brain, lungs, liver and testes/epidimis will be dissected from all rats. Brain, lung and liver tissues will be frozen on dry ice, and stored at -70°C until analysis. Subsequently, brain tissues will be thawed and dissected into cortical, striatal, cerebellar, hippocampal, and brain stem regions, then analyzed for major neurotransmitters levels and metabolite following the methods described in Kim *et al.* (1987). Samples will be randomly selected, thawed, and homogenized in 0.17M perchloric acid (90 mg/ml) using a Polytron homogenizer (GLAS-COL, Terre Haute, IN) then centrifuged at 31,000 g for 30 min at 4°C. The supernatants will be separated and analyzed for concentrations of norepinephrine (NE), epinephrine (E), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA). Blood will be homogenized with an equal volume of 0.34M perchloric acid, and similarly processed. High Performance Liquid Chromatography (HPLC) determinations will be performed with a Dionex Model DX-300 isocratic liquid chromatography unit (Dionex Corp., Sunnyvale, CA) coupled with a pulse electrochemical detector (PED-2). A glassy-carbon working electrode will be set at 0.8 V versus a Ag/AgCl reference electrode. The sensitivity of the detector is maintained between 0.5 and 1.0 nA depending upon the concentration of the neurotransmitters. Separation

by isocratic elution is performed on a C18 reverse phase column preceded by a guard column (Guard-Pak, C18, Waters Association, Milford, MA). The mobile phase is 15% (v/v) methanol in solution of 32 mM citric acid (pH 4.2), 12.5 mM disodium hydrogen orthophosphate, 0.5 mM octyl sodium phosphate, and 0.05 mM EDTA. The mobile phase is filtered through a 0.45 mm filter (Millipore, Bedford, MA) and degassed under a vacuum before use. A flow rate of 1.2 mL/min (2200 pounds/in^2) at ambient temperature is employed. Known amounts of NE, DA, E, DOPAC, HVA, HVA, and 5-HIAA in the range 0.2-20 ng are injected into the HPLC system. The internal standard is 3,4 dihydroxybenzylamine hydrobromide (DHBA, 2.5 ng). All compounds are easily oxidized at 0.8 V versus a Ag/AgCl reference electrode. Each compound will provide a linear response in the 0.2-20 ng range. Lung tissue will be fixed, stained and examined for evidence of pulmonary edema or other histopathological damage. Liver tissue will be evaluated for changes in major enzyme levels. Testicular samples will be analyzed, within 15 min of dissection, for fifteen measures of sperm count, motility and viability, using an automated system.

Statistics

All statistical analyses will be conducted using GB STAT software (Dynamic Microsystems, Inc., Silver Springs, MD). Differences between the means for NTAB test results and for tissue assays will be analyzed utilizing the Student *t*-test. Results of NTAB tests will generally be analyzed with the analysis of variance (ANOVA) statistic, Levenes Test of Homogeneity, and *post hoc* Fischer's LSD (protected *t*-test). NTAB results shown to violate homogeneity of variance requirements, and those containing score maximums ("caps") [Tail Flick Nociception,

One Trial Passive Avoidance, Morris Water Maze and Treadmill Physical Fatigue]. Differences between or among means will be considered significant when $p < 0.05$.

RESULTS

Summary: To date, 32 rats have been exposed for 6 hr/day for 5 days/week for 5 consecutive weeks to JP-8 vapor, estimated at a mean concentration of 1.0 mg/L $\pm$ 10%, and 16 rats have been exposed to room air under similar conditions. The exposure phase of Experiment I will end on 25 September 1998, at which time all rats will be rested for 65 days, then evaluated on 10 NTAB tests for approximately 21 days.

Body Weights and Gross Examinations

Statistical analysis (Student-*t* test), comparing the daily mean weights between air control (n = 16) and JP-8 exposed rats (n = 32) during the 6 wk exposure period indicated a significant ($p < 0.05$) difference. JP-8 exposed rats were significantly lower in weight than air control exposed rats. During the 60 days following the exposures, however, comparison of the daily mean weights of the control and JP-8 exposed rats indicated no significant differences ($p = 0.22$), although the JP-8 rat means remained numerically lower than the control group means. Results for the 60 days post-exposure are presented in graphical form at the conclusion of this update report. All rats have gained weight during the period of exposures, although deprived of food for 6 hr/day. No rats have exhibited signs of physiological irritancy (*i.e.*, respiratory distress, eye area inflammation, fur discoloration, or behavioral signs of pain and distress) and no animals died during the exposures. Two rats exposed to JP-8 have, however, died during the 65 day rest

period. One rat developed significant kidney stones, and the cause of death of the other rat could not be determined.

Exposure Concentrations.

The attached Fig. 1 indicates the daily concentrations ranges of JP-8 vapor, as measured at 30 min intervals by IR spectrometer analysis.

Completion of Experimental Design:

Experiment I: JP-8 Exposures. All neurobehavioral testing will be completed December 21, 1998; animal sacrifices will occur on December 22, 1998.

Experiment II: JP-5 Exposures. JP-5 exposures are planned to begin on October 10, 1998.

Experiment III: Diesel Fuel Exposures. Diesel Fuel exposures are planned to begin on January 20, 1998.

REFERENCES

Alhaider, A. A., and Wilcox, G. L. 1993. Differential roles of 5-hydroxytryptamine1A and 5-hydroxytryptamine1B receptor subtypes in modulating spinal nociceptive transmission in mice. *J. Pharmacol. Exp. Ther.* 265(1):378-385.

Bell, I. R., Rossi III, J., Gilbert, M. E., Kobal, G., Morrow, L. A., Newlin, D. B., Sorg, B. A., and Wood, R. 1997a. Testing the neural sensitization and kindling hypothesis for illness from low levels of environmental chemicals. *Environ. Health Perspect.* 105 Suppl 2:539-547.

Bell, I. R., Schwartz, G. E., Baldwin, C. M., Hardin, E. E., Klimas, N. G., Kline, J. P., Patarca, R., and Song, Z. Y. 1997b. Individual differences in neural sensitization and the role of context in illness from low-level environmental chemical exposures. *Environ. Health Perspect.* 105 Suppl 2:457-466.

Bervoets, K., Rivet, J. M., and Millan, M. J. 1993. 5-HT1A receptors and the tail-flick response. IV. Spinally localized 5-HT1A receptors postsynaptic to serotoninergic neurons mediate spontaneous tail-flicks in the rat. *J. Pharmacol. Exp. Ther.* 264(1):95-104.

Benignus, V. A. 1981. Health effects of toluene: a review. *Neurotoxicol.* 2(3):567-588.

Bonhomme, N., De Deurwaerdere, P., Le Moal, M., and Spampinato, U. 1995. Evidence for 5-HT4 receptor subtype involvement in the enhancement of striatal dopamine release induced by serotonin: a microdialysis study in the halothane-anesthetized rat. *Neuropharmacol.* 34(3):269-279.

Bruner, R. H., Kinkead, E. R., O'Neill, T. P., Flemming, C. D., Mattie, D. R., Russell, C. A., and Wall, H. G. 1993. The toxicologic and oncogenic potential of JP-4 jet fuel vapor in rats and mice: 12-month intermittent inhalation exposure. *Fundam. Appl. Toxicol.* 20(1): 97-110.

Burbacher, T. M. 1993. Neurotoxic effects of gasoline and gasoline constituents. *Environ. Health Perspect.* 101 Suppl 6:133-141.

Bushnell, P. J., Kelly, K. L., and Crofton, K. M. 1994. Effects of toluene inhalation on detection of auditory signals in rats. *Neurotoxicol. Teratol.* 16(2):149-160.

Chojnacka-Wojcik, E. 1992. Involvement of dopamine autoreceptors in the hypoactivity induced by 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) in mice. *Pol. J. Pharmacol. Pharm.* 44(2):135-146.

Contreras, C. M., Gonzalez-Estrada, T., Zarabozo, D., and Fernandez-Guardiola, A. 1979. Petit mal and grand mal seizures produced by toluene or benzene intoxication in the cat. *Electroencephalogr. Clin. Neurophysiol.* 46(3):290-300.

Cooper, J. R., and Mattie, D. R. 1996. Developmental toxicity of JP-8 jet fuel in the rat. *J. Appl. Toxicol.* 16(3):197-200.

CRC Handbook of Chemistry and Physics, CRC Press, Cleveland, 1997.

Cunningham, K. A., Paris, J. M., and Goeders, N. 1992. Chronic cocaine enhances serotonin autoregulation and serotonin uptake binding. *Synapse* 11(2):112-123.

De Deurwaerdere, P., Bonhomme, N., Lucas, G., Le Moal, M., and Spampinato, U. 1996. Serotonin enhances striatal dopamine outflow in vivo through dopamine uptake sites. *J. Neurochem.* 66(1):210-215.

Dossing, M., Loft, S., and Schroeder, E. 1985. Jet fuel and liver function. *Scand. J. Work Environ. Health* 11(6):433-437.

Forkman, B. A., Ljungberg, T., Johnson, A. C., Nylen, P., Stahle, L., Hoglund, G., and Ungerstedt, U. 1991. Long-term effects of toluene inhalation on rat behavior. *Neurotoxicol. Teratol.* 13(5):475-481.

Fujii, T., Yoshizawa, M., Nakai, K., Fujimoto, K., Suzuki, T., and Kawashima, K. 1997. Demonstration of the facilitatory role of 8-OH-DPAT on cholinergic transmission in the rat hippocampus using in vivo microdialysis. *Brain Res.* 761(2):244-249.

GB STAT Statistical Software System, Version 3.1. Dynamic Microsystems, Inc., Silver Springs, MD.

Geyer, M. A. 1996. Serotonergic functions in arousal and motor activity. *Behav. Brain Res.* 73(1-2):31-35.

Geyer, M. A., and Braff, D. L. 1987. Startle habituation and sensorimotor gating in schizophrenia and related animal models. *Schizophr. Bull.* 13(4):643-668.z

Gralewicz, S., Wiaderna, D., and Tomas, T. I. 1995. Development of spontaneous, age-related nonconvulsive seizure electrocortical activity and radial-maze learning after exposure to m-xylene in rats. *Int. J. Occup. Med. Environ. Health* 8(4):347-360.

Gralewicz, S., Wiaderna, D., Tomas, T., and Rydzynski, K. 1997. Behavioral changes following 4-week inhalation exposure to pseudocumene (1,2,4-trimethylbenzene) in the rat. *Neurotoxicol. Teratol.* 19(4):327-333.

Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules. Daimay May-Lin *et. al* (eds.), Academic Press, Boston, 1991.

Harris, D. T., Sakiestewa, D., Robledo, R. F., and Witten, M. 1997. Immunotoxicological effects of JP-8 jet fuel exposure. *Toxicol. Ind. Health* 13(1):43-55.

Hinners R.G., Burkhart J.Y., and Punte E.C. 1968. Animal inhalation exposure chambers. *Arch Environ Health* 16:194-206.

Ikeda, T., Katakura, Y., Kishi, R., and Miyake, H. T. 1993. Acute neurobehavioral effects of co-inhalation of toluene and n-hexane on schedule-controlled behavior in rats. *Environ. Res.* 63(1):70-81.

Johansson, C. E., Meyerson, B. J., and Hoglund, A. U. 1990. The long-term effects of 8-hydroxy-2-(di-n-propyl-amino) tetralin (8-OH-DPAT) on copulatory and exploratory behaviour in male rats. *Eur. J. Pharmacol.* 178(1):1-9.

Kim, C., Speisky, M. B., Kharouba, S. N. 1987. Rapid and sensitive method for measuring norepinephrine, dopamine, 5-hydroxytryptamine and their major metabolites in rat brain by high-performance liquid chromatography. Differential effect of probenecid, haloperidol and yohimbine on the concentrations of biogenic amines and metabolites in various regions of rat brain. *J. Chromatogr.* 386:25-35.

Kinkead, E. R., Wolfe, R. E., Bunger, S. K., and Leahy, H. F. 1992. The acute toxicity evaluation of a low-temperature hydraulic fluid. *Am. Ind. Hyg. Assoc. J.* 53(3):163-168.

Knave, B., Persson, H. E., Goldberg, J. M., and Westerholm, P. S. 1976. Long-term exposure to jet fuel: an investigation on occupationally exposed workers with special reference to the nervous system. *Scand. J. Work Environ. Health.* 2(3):152-164.

Knave, B., Olson, B. A., Elofsson, S., Gamberale, F., Isaksson, A., Mindus, P., Persson, H. E., Struwe, G., Wennberg, A., and Westerholm, P. S. 1978. Long-term exposure to jet fuel. II. A cross-sectional epidemiologic investigation on occupationally exposed industrial workers with special reference to the nervous system. *Scand. J. Work Environ. Health.* 4 (1):19-45.

Knave, B., Mindus, P., and Struwe, G. S. 1979. Neurasthenic symptoms in workers occupationally exposed to jet fuel. *Acta Psychiatr. Scand.* 60(1):39-49.

Kostrzewa, R. M., Brus, R., Kalbfleisch, J. H., Perry, K. W., and Fuller, R. W. 1994. Proposed animal model of attention deficit hyperactivity disorder. *Brain Res. Bull.* 34(2):161-171.

Kostrzewa, R. M. 1995. Dopamine receptor supersensitivity. *Neurosci. Biobehav. Rev.* 19(1): 1-17.

MacEwen, J. D. and Vernot, E. H. 1984. *Toxic Hazards Research Unit Annual Technical Report: Govt Reports Announcements & Index (GRA&I)* Issue 04.

MacMahon, K. L., Eggers, J., Wolfe, R. E., Leahy, H. F., Narayanan, L., Rossi III, J., Nordholm, A., Ritchie, G., and Witzmann, F. 1998. Evaluation of exposure to pyridostigmine bromide, diethyltoluamide, JP-4 jet fuel and stress on male Sprague-Dawley rats. *Toxic Hazards Research Unit Annual Technical Report: Govt Reports Announcements & Index (GRA&I)* AL/OE-TR-1997.

Macys, D. A., Carpenter, R. L., Risher, J. F., Vinegar, A., Dodd, D. E., and Wall, H. G. 1992. Results of a workshop on health effects of crude oil exposures related to Operation Desert Storm. *Naval Medical Research Institute, Government Publication*, NMRI 92-04.

Mattie, D. R., Marit, G. B., Flemming, C. D., and Cooper, J. R. 1995. The effects of JP-8 jet fuel on male Sprague-Dawley rats after a 90-day exposure by oral gavage. *Toxicol. Ind. Health* 11(4):423-435.

Mattie, D. R., Marit, G. B., Flemming, C. D., Sterner, T. R., and Cooper, J. R. 1996. The effects of JP-8 jet fuel on female Sprague-Dawley rats after a 21-week exposure by oral gavage. The Toxicologist, 30(1):44.

Mattie, D.R., Alden, C.L., Newell, T.K., Gaworski, C.L., and Flemming, C.D. 1991. *Toxic. Path.* 19:77.

Miyagawa, M., Honma, T., and Sato, M. 1995. Effects of subchronic exposure to toluene on working and reference memory in rats. *Neurotoxicol. Teratol.* 17(6):657-664.

Niklasson, M., Stengard, K., and Tham, R. 1995. Are the effects of toluene on the vestibulo- and opto-ocular motor system inhibited by the action of $GABA_B$ antagonist CGP 35348. *Neurotoxicol. Teratol.* 17(3):351-371.

Nisijima, K., and Ishiguro, T. 1995. Cerebrospinal fluid levels of monoamine metabolites and gamma-aminobutyric acid in neuroleptic malignant syndrome. *J. Psychiatr. Res.* 29(3): 233-244.

Odkvist, L. M., Arlinger, S. D., Edling, C., Larsby, B., and Bergholtz, L. M. 1987. Audiological and vestibulo-oculomotor findings in workers exposed to solvents and jet fuel. *Scand. Audiol.* 16(2):75-81.

Panksepp, J., Nelson, E., and Bekkedal, M. 1997. Brain systems for the mediation of social separation-distress and social-reward. Evolutionary antecedents and neuropeptide intermediaries. *Ann. N.Y. Acad. Sci.* 807:78-100.

Pfaff, J., Parton, K., Lantz, R. C., Chen, H., Hays, A. M., and Witten, M. L. 1995. Inhalation exposure to JP-8 jet fuel alters pulmonary function and substance P levels in Fischer-344 rats. *J. Appl. Toxicol.* 15 (4):249-256.

Porter, H.O. 1990. Aviators intoxicated by inhalation of JP-5 fuel vapor. *Aviat. Space Environ. Med.* 61(7):654-656.

Pryor, G. T. 1991. A toluene-induced motor syndrome in rats resembling that seen in some human solvent abusers. *Neurotoxicol. Teratol.* 13(4):387-440.

Rahill, A. A., Weiss, B., Morrow, P. E., Frampton, M. W., Cox, C., Gibb, R., Gelein, R., Speers, D., and Utell, M. J. 1996. Human performance during exposure to toluene. *Aviat. Space Environ. Med.* 67(7):640-671.

Rigdon, C. C., and Witherspoon, J. K. 1992. 5-Hydroxytryptamine 1a receptor agonists block prepulse inhibition of acoustic startle reflex. *J. Pharmacol. Exp. Ther.* 263(2):486-493.

Ritchie, G.D., Ademujohn, C.Y., MacInturf, S., Hulme, M.E., McCool, C. Nordholm, A.F., Rossi III, J., MacMahon, K., Leahy, H., and Wolfe, R.E. Repeated exposure of rats to JP-4 vapor induces changes in neurobehavioral capacity and 5-HT/5-HIAA levels. *J Toxicol Environ Health* Submitted for publication.

Ritchie, G. D., Rossi III, J., Macys, D. A., and Still, K. R. 1995. Application of the NMRI/TD Neurobehavioral Screening Battery to combustion toxicology. In *Fire and Polymers II: Materials and tests for Hazard Prevention*, ed. G. Nelson, pp 344-366. Washington, D.C.: ACS Books.

Robinson, T. E., and Becker, J. B. 1986. Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. *Brain Res.* 396(2):157-198.

Sipes, T. A., and Geyer, M. A. 1995. 8-OH-DPAT disruption of prepulse inhibition in rats: reversal with (+)WAY 100,135 and localization of site of action *Psychopharmacology Berl.* 117(1):41-81.

Smith, E. A., Kimmel, E. C., English, J. H., Bowen, L. E., Reboulet, J. E., and Carpenter, R. L. 1997. Evaluation of the respiratory tract after acute exposure to a pyrotechnically generated aerosol fire suppressant. *J. Appl. Toxicol.* 17(2):95-103.

Sorg, B. A., Hooks, M. S., Kalivas, P. W. 1994. Neuroanatomy and neurochemical mechanisms of time-dependent sensitization. *Toxicol. Ind. Health* 10(4-5):369-386.

Sorg, B. A., Willis, J. R., Nowatka, T. C., Ulibarri, C., See, R. E., and Westberg, H. H. 1996. Proposed animal neurosensitization model for multiple chemical sensitivity in studies with formalin. *Toxicol* 111(1-3):135-145.

Struwe, G., Knave, B., and Mindus, P. S. 1983. Neuropsychiatric symptoms in workers occupationally exposed to jet fuel--a combined epidemiological and casuistic study. *Acta Psychiatr. Scand.* 67 (Suppl. 303):55-67.

Struwe, G., and Wennberg, A. 1983. Psychiatric and neurological symptoms in workers occupationally exposed to organic solvents - results of a differential epidemiological study. *Acta Psychiatr. Scand.* 67 (Suppl. 303):68-80.

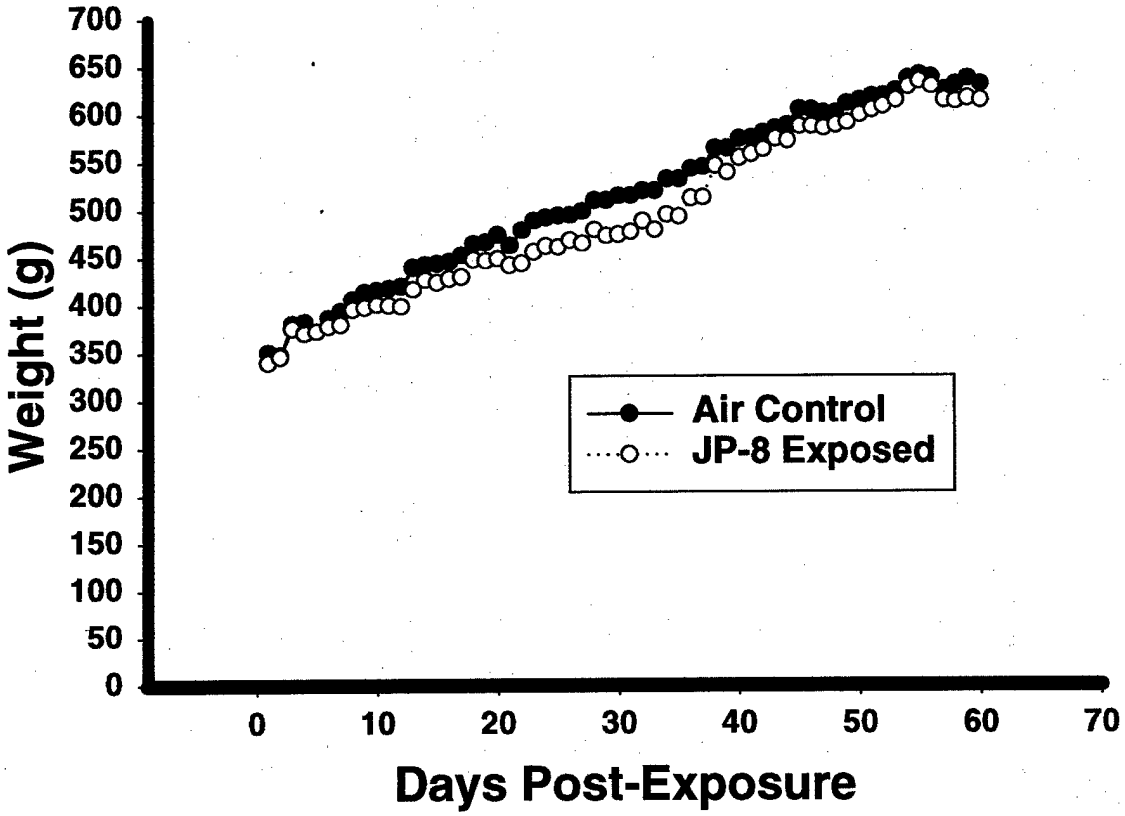
Swerdlow, N. R., Mansbach, R. S., Geyer, M. A., Pulvirenti, L., Koob, G. F. and Braff, D. L. 1990. Amphetamine disruption of prepulse inhibition of acoustic startle is reversed by depletion of mesolimbic dopamine. *Psychopharmacol. Berl.* 100(3):413-446.

Varty, G. B., and Higgins, G. A. 1995. Examination of drug-induced and isolation-induced disruptions of prepulse inhibition as models to screen antipsychotic drugs. *Psychopharmacol. Berl.* 122(1):15-26.

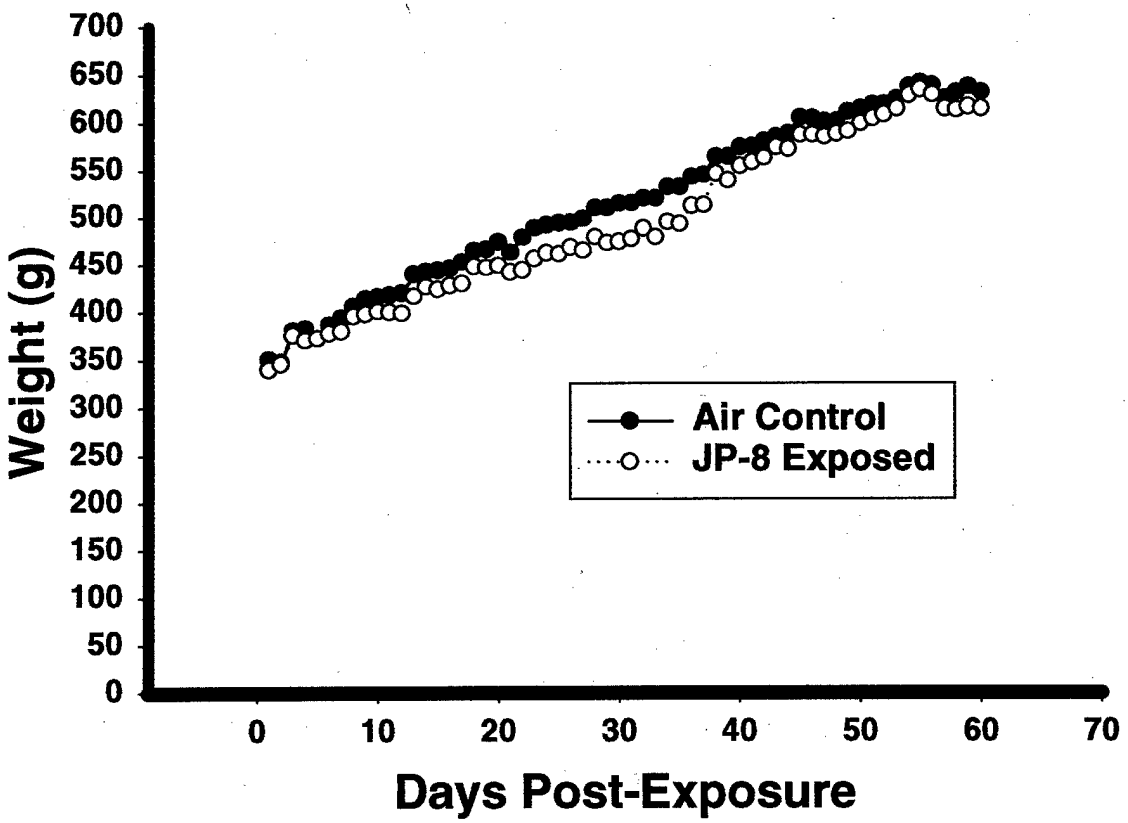
von Euler, G., Ogren, S. O., Li, X. M., Fuxe, K., and Gustafsson, J. A. 1993. Persistent effects of subchronic toluene exposure on spatial learning and memory, dopamine-mediated locomotor activity and dopamine D2 agonist binding in the rat. *Toxicology* 77(3): 223-232.

Wolfe, R. E., Kinead, E. R., Feldmann, M. L., Leahy, H. F., and Jederberg, W. W. 1997. Acute toxicity evaluation of JP-8 Jet Fuel and JP-8 Jet Fuel containing additives. *Govt. Reports Announcements & Index (GRA&I),* Issue 09.

Mean Rat Weights 60 Days Post-Exposure



Mean Rat Weights 60 Days Post-Exposure



REPEATED EXPOSURE OF RATS TO JP-4 VAPOR INDUCES CHANGES IN NEUROBEHAVIORAL CAPACITY AND 5-HT/5-HIAA LEVELS

Alan F. Nordholm, John Rossi III

Naval Health Research Institute Detachment-Toxicology, Wright-Patterson Air Force Base, Ohio, USA

Glenn D. Ritchie, Shawn McInturf, Mary E. Hulme, Candice McCool, Lathe Narayanan

Geo-Centers, Inc., Wright-Patterson Air Force Base, Ohio, USA

Kathleen L. MacMahon, Jeffrey Eggers

Occupational Toxicology Branch, Wright-Patterson Air Force Base, Ohio, USA

Harry F. Leahy, Robin E. Wolfe

ManTech Environmental Technology, Inc., Wright-Patterson Air Force Base, Ohio, USA

Thirty-two Sprague-Dawley rats were exposed for 6 h/d for 14 consecutive days to JP-4 jet fuel vapor (2 mg/L) or room air control conditions. Following a 14- or 60-d recovery period, rats completed a battery of 8 tests selected from the Navy Neurobehavioral Toxicity Assessment Battery (NTAB) to evaluate changes in performance capacity. Exposure to JP-4 vapor resulted in significant changes in neurobehavioral capacity on several tests that varied as a function of the duration of the recovery period. Rats were evaluated for major neurotransmitter and metabolite levels in five brain regions and in the blood serum. Levels of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were shown to be significantly elevated in several brain regions as well as in the blood serum in the vapor-exposed groups. Results of the rat study are compared to previously reported neurobehavioral evaluations of European manufacturing personnel exposed chronically to jet fuel vapor.

There is increasing evidence that repeated exposure to doses below threshold limit values (TLV) of one or more of a diversity of chemical toxicants can result in persisting changes in neurobehavioral capacity (Burbacher, 1993). These changes can range from acute performance-reducing physiological irritancy effects to severe persisting changes in

Received 14 August 1998; sent for revision 17 September 1998; accepted 6 November 1998.

This research was funded by the U.S. Army Medical Research and Materiel Command (USAMRMC). The opinions and assertions contained herein are those of the authors, and are not to be construed as those of the U.S. Army or the military service at large. The experiments reported herein were conducted according to the principles set forth in the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council, DHHS, Publication No. (NIH) 86-23 (1996).

Address correspondence to Dr. Glenn D. Ritchie, NHRCITD, 2612 Fifth St., Building 433, Wright-Patterson Air Force Base, OH 45433, USA. E-mail: gritchie@navy.al.wpafb.af.mil

Journal of Toxicology and Environmental Health, Part A, 56:471–499, 1999

0098-4108/99 $12.00 + .00

Enclosure (1)

physical or mental health. The exposure effects may, as in the cases of idiopathic environmental intolerance (IEI; formerly multiple chemical sensitivity) or sick building syndrome (SBS), be debilitative to the subject in the most severe case, or may in lesser cases remain undetected without evaluation using specialized neurobehavioral tests. The IEI syndrome may, for example, occur following a single or repeated exposure to one or more chemical toxicants, may begin from days to months postexposure, may increase in severity over time without development of reliable medical biomarkers, may generalize to include adverse reactions to a diversity of previously neutral chemical stimuli, and may selectively impact only a small percentage of the exposed population (Bell et al., 1997a, 1997b).

Repeated exposure to jet fuel vapor is a potential problem for fuel manufacturing, transporting, and handling workers, as well as for aircraft manufacturing, maintenance, and operational personnel in both military and civilian sectors (Harris et al., 1997). It is estimated that the U.S. military is currently consuming nearly 2.2 billion gallons of jet fuels per year. Before 1992, the U.S. military utilized primarily JP-4 jet fuel and JP-5 (U.S. Navy), but since 1992 has replaced virtually all JP-4 applications with JP-8, and is in the process of replacing many JP-5 applications. Jet fuels, containing potentially neurotoxic compounds including benzene, toluene, xylene, and *n*-hexane, as well as numerous other short- and long-chain aromatic and aliphatic hydrocarbons (Mattie et al., 1991), have recently been combined with thermal stability additives whose neurobehavioral toxicity potential may be unknown (Wolfe et al., 1997). In terms of volatility and the concentration (Leahy, 1996) of neuroactive volatile organic chemicals (VOCs) it has been shown generally that JP-4 > JP-5 ≥ JP-8, and that hydrocarbons above C14 existed only in very low concentrations in any of the vapors. Because half-lives for VOC components of jet fuel (i.e., toluene and xylene) may vary from several hours to nearly 2 d, it is probable that repeated daily exposures to jet fuels may result in additive VOC concentrations in body tissues that exceed those expected from a single exposure.

Acute laboratory exposure to jet fuel vapor (JP-8) in humans has been reported only to be slightly irritating to the skin, with a weak dermal sensitizing potential (Kinkead et al., 1992). However, a report was published (Porter, 1990) describing effects of the accidental exposure to JP-5 vapor of two U.S. aviators during acrobatic flight. The pilots reported burning eyes, nausea, fatigue, impairment of eye-hand coordination, euphoria, and memory deficits. Long-term toxicity testing of jet fuel vapor and aerosol (JP-8) in rats (exposure to 0–1000 mg/m^3 for 90 d) indicated limited toxicity and no tumor formation (Mattie et al., 1991, 1995). A developmental toxicity study of JP-8 vapor exposure indicated that the fuel is not a teratogen in the rat (Cooper & Mattie, 1996), although daily oral gavage dosing (0–3000 mg/kg/d) of male rats in this study resulted in decreased body weights, nephropathy, gastritis, perianal dermatitis, and elevated liver

enzymes. Dossing et al. (1985), studying the effect in fuel-filling attendants of repeated exposure to jet fuel, reported significantly enhanced antipyrine clearance, indicating fuel-related induction of hepatic metabolism. Pfaff et al. (1995), exposing rats to 520 mg/m^3 JP-8 vapor/aerosol for 1 h/d for 7 d, reported an increase in dynamic lung compliance and pulmonary resistance, with a decrease in substance P concentrations from the bronchoalveolar lavage fluid (BALF). Finally, it has been recently shown (Harris et al., 1997) that nose-only exposure of mice for 1 h/d for 7 d to varying concentrations (100–2500 mg/m^3) of vaporized/aerosolized JP-8 jet fuel resulted in significant immunotoxicity, as evidenced by reductions in splenic and thymic weights, loss of viable immune cell numbers, and a compromised immune function.

Perhaps due to the minimal carcinogenicity, tumorigenesis, and developmental toxicity risks from acute or chronic jet fuel exposure, and because documented physiological consequences have been limited to non-central nervous system tissues (Bruner et al., 1993) there have been few published studies targeting neurobehavioral toxicity in humans, and virtually none using animals (Macys et al., 1992). Several studies (Knave et al., 1976, 1978, 1979; Struwe & Wennberg, 1983; Struwe et al., 1983) have, however, reported the occurrence of neurasthenia (fatigue, anxiety, mood disorders, memory difficulties, and psychosomatic symptoms), psychasthenia, polyneuropathy, changes in sensorimotor speed and attention, electroencephalograph (EEG) abnormalities, and possible sexual dysfunction in civilian and military aircraft workers. These workers were typically exposed to jet fuel vapor/aerosols of 250–300 mg/m^3 in the manufacturing environment for from 4 to 32 yr.

A relatively larger number of studies have been published investigating the neurobehavioral consequences of brief or chronic exposure of humans or animals to component chemicals of hydrocarbon jet fuels, including toluene (Benignus, 1981; Struwe & Wennberg, 1983; Odkvist et al., 1987; Pryor, 1991; Forkman et al., 1991; Ikeda et al., 1993; von Euler et al., 1993; Bushnell et al., 1994; Miyagawa et al., 1995; Niklasson et al., 1995; Rahill et al., 1996), xylene (Gralewicz et al., 1995), and benzene (Contreras et al., 1979; Gralewicz et al., 1997). Human or animal effects from these exposures include vertigo and dizziness, headache, body and joint pain, cardiac palpitations, nausea and gastritis, fatigue, tremor, vigilance deficits, increased reaction time, motor incoordination, euphoria, mild hallucinations, emotional hostility, memory deficits, insomnia, reduced ability to concentrate, and increased latency of responding, without reductions in accuracy on complex performance tests. In the extreme case, Contreras et al. (1979) reported progressive development of absence-like seizures, subclinical seizures, and generalized seizures in cats exposed repeatedly to toluene or benzene, hypothesizing a kindling mechanism.

The present study investigated the short-term and longer term neurobehavioral consequences of repeated exposure of rats to levels of JP-4 jet

fuel vapor insufficient to induce observable physiological irritancy effects. Although JP-8 has replaced JP-4 as the major fuel source used by the U.S. military (Harris et al., 1997), JP-4 vapor exposures were analyzed in this study to simulate the chronic exposures encountered by jet fuel manufacturing personnel and exposed military veterans enlisted before 1992. NTAB tests were selected from the larger battery for their ability to identify in exposed rats possible performance deficits with similarity to those identified (Knave et al., 1976, 1978, 1979; Struwe & Wennberg, 1983; Struwe et al., 1983) in European fuel workers (e.g., fatigue, polyneuropathy, changes in sensorimotor speed), or in some military personnel (Fiedler et al., 1996; Jamal et al., 1996; Haley et al., 1997a, 1997b) assigned to combat theaters where repeated exposure to JP-4 or JP-8 jet fuel has been reported (e.g., malagias, muscle weakness, fatigue and postexertional malaise, photophobia, altered pain threshold, and altered acoustic startle/habituation response). A battery of brain and blood serum neurotransmitter analyses were conducted to identify reliable biomarkers in exposure groups in which a neurobehavioral change was observed.

MATERIALS AND METHODS

Animals

Thirty-two male Sprague-Dawley rats (weighing 250–325 g at the onset of exposures) [Crl:CD (BR)] (50 d of age upon receipt) were procured from Charles River Breeding Laboratories, Raleigh, NC. Prior to neurobehavioral testing rats were pair housed in hanging polycarbonate shoebox cages with cellulose fiber contact bedding (Cell-Sorb Plus, A. W. Products, Inc., New Philadelphia, OH). During neurobehavioral testing procedures, rats were individually housed in similar cages. Fresh pelleted food (Purina Formulab 5002, Purina Mills, Inc., St. Louis, MO) and fresh conditioned (reverse osmosis) water were available ad libitum. Room air temperature and humidity was maintained at 23 ± 2°C and 55 ± 10%, respectively. Rodent cage racks were located within Bio-Clean air mass displacement units that provided a constant supply of HEPA-filtered air. Electronically controlled full spectrum fluorescent light was provided on a 12:12 h light:dark cycle; all exposures and testing occurred during the light cycle.

JP-4 Jet Fuel

The jet fuel exposed animals received 2 mg/L ± 10% JP-4 vapor supplied by Wright Laboratory, Wright-Patterson Air Force Base, OH. Gas chromatographic analysis of the fuel was performed.

Preexposure Conditions

Upon receipt, all rats were quarantined for 14 d. During the final 3 d of quarantine and for the next 2 d, each rat was handled for 10 min/d. Following handling procedures, rats were assigned randomly (n = 16) to either the JP-4 vapor (V) or air control (AC) exposure conditions.

Exposure Conditions

Groups of 16 rats each were exposed to V or AC atmospheres for 6 h/d for 14 consecutive days in one of two 700-L Toxic Hazards Research Unit (THRU) whole-body exposure chambers (Smith et al., 1997). During the exposures animals were deprived of both food and water. Rats were assigned using a computer randomization program to 1 of 32 possible housing locations within the assigned chamber on each day of exposure to minimize effects from possible regional differences in JP-4 vapor concentration within the chamber. Each housing location consisted of a wire mesh cage (in an 8-cage housing rack), 17 cm (l) × 12 cm (w) × 17 cm (h), designed to maximize vapor flowthrough. AC rats in one THRU chamber experienced room air flowing at 6 ft^3/min (cfm). V rats in a second THRU chamber experienced room air mixed with JP-4 vapor at a concentration of 2 mg/L ± 10%, generated by metering approximately 1.8 mL fuel/min via a Buchler multistaltic pump (model 426-2000, Buchler Instruments, A Labconco Co., Lexena, KA) into the top of a counterflow (3 cfm), heated (45°C) evaporator tower. The output was delivered from a modified DeVilbiss nebulizer (model 099HD, Somerset, PA) at a dynamic flow rate of 6 cfm. Daily measurements of relative humidity, temperature, and barometric pressure were made at the time of exposure and were excluded as significant causes of variability (±10%) in JP-4 vapor concentrations. Measurement of the fuel mass concentration during the 14-d study was continuously quantified using the infrared (IR) absorbance band between 3.4 and 3.5 μm (Miran 1A, Foxboro Analytical, Wilks Infrared Center, South Norwalk, CT) calibrated with known mass concentrations of hexane. Less than 2% of the input fuel was recovered as flow-by or in-line condensate. No aerosol was detected when chamber atmospheres were sampled with Gelman 25-mm, extra thick glass fiber filters (Gelman Sciences, Ann Arbor, MI). Gas chromatographic analysis of the fuel, fuel vapor, and spent fuel were also performed. The chamber vapor chromatographs resembled closely those of the original jet fuel, with only a slight shift to the lighter fractions of JP-4 jet fuel components. The spent fuel was essentially devoid of jet fuel components lighter than C11 (undecane) hydrocarbons. Auditory volume within the THRU chambers related to the inflow and outflow of control or test atmospheres averaged 85 ± 2 dB.

Postexposure Conditions

Following each daily 6-h exposure, rats were removed from the inhalation exposure housing cages and returned to the home cages where ad libitum food and water were available. Each animal was weighed and inspected by the veterinary services staff for evidence of physiological irritancy or other medical debilitation.

Postexposure Resting

Following the completion of inhalation exposures, 50% of the rats in the AC and V exposure groups were randomly assigned to either a 14-d

(short recovery group, SRG) or 60-d (long recovery group, LRG) group. During the recovery periods, rats were pair housed by random assignment in the home cages with ad libitum access to food and water, and were weighed and examined on a daily basis.

NTAB Testing

Following either the 14- or 60-d recovery, rats were evaluated on each of 8 neurobehavioral tests selected from the NTAB (Ritchie et al., 1995). Selection of NTAB tests from the larger battery was based on the assumed capacity of individual tests to identify neurobehavioral deficits in rats similar to those observed previously in human aircraft workers chronically exposed to jet fuel vapor (Knave et al., 1976, 1978, 1979; Struwe & Wennberg, 1983; Struwe et al., 1983). All rats experienced the identical NTAB tests in the same order of presentation (i.e., the forelimb grip strength test always preceded the photosensitivity test, etc.). For each individual test, the AC and V exposed rats were evaluated in randomized order, with the experimenters blinded to the exposure history of individual subjects. Completion of NTAB subbattery testing required approximately 21 d per animal group. Tests are listed in Table 1.

DESCRIPTION OF NEUROBEHAVIORAL TESTS

Test 1. Forelimb Grip Strength (FGS) Rat forelimb grip strength is a well-validated method for assessing the neuromotor effects of environmental and psychopharmacological agents (Meyer et al., 1979). Hand-held rats were allowed to grasp the mesh pull screen of a standard grip strength meter for rats (1027S-D-30; front limb testing unit only, Columbus Instruments, Columbus, OH). When a firm grip was observed, the rat was slowly pulled (held at the base of the tail) at a constant force in a linear direction parallel to the floor surface at a speed of approximately 3 cm/s until release of both forepaws was observed. At this point, the digital output of the grip strength meter (peak force, kg) was recorded, and another trial was begun. Each animal was tested for 3 trials, with an intertrial

TABLE 1. NTAB Tests

Test	Acronym	NTAB test
1	FGS	Forelimb grip strength
2	PS	Photosensitivity
3	ARAS	Appetitive reinforcer approach sensitization
4	TLA	Total locomotor activity
5	ASR	Acoustic startle response
6	PPI	Prepulse inhibition of ASR
7	TFR	Tail flick response
8	TPF	Treadmill physical fatigue

stimulus approach areas. These etched semicircles were used to define rat approach to the stimulus display areas containing the hamburger meat versus similar approach to the nonbaited corners. A 15-cm-radius circle was etched from the geometric center of the apparatus to locate initial placement of the rats within the apparatus. The apparatus was rotated 180° after every 2 consecutive trials to counterbalance possible rat directional preferences, the 2 grating barriers defining the baited stimulus presentation areas were switched with those defining the nonbaited stimulus presentation areas after every fourth consecutive trial, and the apparatus was thoroughly washed with 100% ethanol between trials to minimize odor cues. To begin each trial, the rat was placed by one experimenter into the center circle. One of two independent observers recorded the number of seconds (of a 600-s maximum) that each individual rat spent with at least two forepaws on or across the semicircular lines (in the stimulus approach zones) etched near the two baited stimulus display areas, while the second observer recorded the number of seconds spent in the stimulus approach zones near the two nonbaited stimulus display areas. Room illumination was provided by a 25-W red bulb suspended over the apparatus.

Test 4. Total Locomotor Activity (TLA) Randomly selected groups of four rats each were evaluated for total horizontal and vertical locomotor activity. Rats were placed individually in one of four 17 × 17-in Opto-Varimex animal activity meters (model Opto-Varimex-3, Columbus Instruments, Columbus, OH) for 30 min. The only illumination in the experimental room was provided by a 40-W red bulb suspended near the activity meters. Through use of autotrack features (two 8 × 8 infrared light beam matrices for horizontal plane motion and vertical rearing) the following measures were automatically recorded:

1. Horizontal beam interruptions (total number/30 min).
2. Vertical beam interruptions (total number/30 min).

Test 5–6. Acoustic Startle Response–Prepulse Inhibition (AS/PPI) Randomly selected groups of four rats each were placed within four acoustic startle response system (model SR-Lab, San Diego Instruments, San Diego, CA) restraint tubes (250–500 g rat model) housed within four standard sound and light attenuation chambers. The chambers were illuminated with 40-W standard light bulbs. All boxes were routinely calibrated and equated for startle platform sensitivity (SR-LAB Calibrator Standardization Units) and startle tone intensity (standard audiometer). Rats were placed within the restraint tubes such that the head was oriented in the direction of an audio speaker located approximately 3 cm from the restraint tube door. Stimulus intensities listed below reflect the mean measured sound intensity reaching the subjects within the startle tube with the forward and rear doors in the closed position. Each animal received 8 discrete auditory startle sessions (10 startles per session) dur-

interval (ITI) of approximately 30 s. The dependent measure was the mean peak grip strength force (kg) calculated for the three trials.

Test 2. Photosensitivity Test (PS) For the PS test, each rat was individually tested for photosensitivity in a two-chamber shuttle box (Reflex-16 Shuttle Box, Columbus Instruments, Columbus, OH) for a period of 600 s. A similar test, utilizing a circular light–dark preference alley, is described by van der Staay and Blokland (1996) as a measure of light aversion, or alternately, of dark preference in four rat strains. The shuttle box, with black Plexiglas flooring, sides, and lid (except for an observation port on the lighted side), was equally divided into lighted and non-illuminated chambers separated by a Plexiglas divider, with a 8 × 10 cm door that could be manually lifted or closed. The floor of the apparatus consisted of 3-mm-diameter metal grid bars spaced at approximately 1 cm. A single 40-W standard light bulb, mounted above the white, translucent ceiling of the illuminated (right) chamber, provided the only illumination in the apparatus. Rats were placed initially in the lighted chamber, with the animal facing the rear wall of the apparatus, and the door dividing the chambers was immediately opened. The number of seconds (of a 600-s maximum) that each rat spent in the lighted versus the dark chamber was the dependent measure.

Test 3. Appetitive Reinforcer Approach Sensitization (ARAS) The ARAS test, described by Rossi III et al. (1996), is a newly devised test hypothesized to identify long-term sensitization of dopaminergic systems. The singly-housed rats were provided access to 7–10 g raw hamburger meat at approximately 1600 h on each of 2 d separated by a 24-h period. The amount of hamburger meat consumed was calculated at 800–900 h of the following day by weighing any hamburger remaining in the home cage. Rats were then tested for appetitive reinforcer approach sensitization 36–48 h following the second raw hamburger meat presentation. Rats were individually placed for 600 s each within a 30 × 90 × 30 (high) cm non-illuminated rectangular chamber constructed of ½-in clear Plexiglas sides and a ½-in clear Plexiglas floor covered on the bottom side with black contact paper. In each of the 4 corner junctions of the chamber, a 15 cm wide × 30 cm high rectangular piece of white plastic, fluorescent light fixture grating (1.2 cm × 1.2 cm plastic mesh) was mounted to form, with the side walls of the apparatus, a triangular-shaped stimulus display area. The removable grating barrier permitted visual and olfactory recognition to the rat of the hamburger meat stimulus without tactile access. Behind 2 stimulus holding areas diagonally opposite of the rectangular box was placed 10 g of raw hamburger meat on 4-cm-diameter plastic weighing dishes set on 4-cm-high cylindrical, clear Plexiglas pedestals. Within the remaining two stimulus display areas were placed identical lids and pedestals containing no hamburger meat stimulus. Concentric semicircles (0.5 cm wide) with a 15-cm radius were etched into the bottom of the Plexiglas flooring, using each corner of the apparatus as the semicircle's center, to define 4

ment level was set at 0.45–0.5 mA (apparatus setting 9.0) for 100 ms, at a frequency of 9 negative reinforcements/s (apparatus setting 9.0). During the pretraining period each animal experienced several negative reinforcements related to entry into the trough. Twenty-four hours following the pretraining, randomly selected groups of 2 rats each were placed on the 2 lanes of the treadmill and allowed to walk for up to 45 min (2700 s) at a lane speed of 0.250 m/s, with a lane inclination of 15°, and negative reinforcement intensity of 0.45–0.50 mA (duration 100 ms; rate of 9 negative reinforcements/s). The dependent measure of physical fatigue was latency to the fifth entry of two or more limbs into the trough area.

Biomarker Testing

Within 24–48 h of the completion of NTAB testing, rats were sacrificed in randomized order by CO_2 overdose. At each necropsy, blood samples were taken from the vena cava for subsequent analysis. Major tissue samples (heart, lungs, liver, kidneys, spleen, stomach, duodenum, jejunum, ileum, colon, testes, thymus, femur bone marrow, sciatic nerve, skeletal muscle, skin, cervical lymph nodes, thyroids, parathyroids, adrenals, pituitary, and cervical and lumbar vertebrae with spinal cord) were removed from all rats and preserved in a 10% buffered formalin solution. Following sacrifice, brain tissues were immediately removed from 6 randomly selected rats in each treatment group, frozen on dry ice, and stored at –70°C until analysis. Subsequently, brain tissues were thawed and dissected into cortical, striatal, cerebellar, hippocampal, and brainstem regions and analyzed for major neurotransmitters levels following the methods described in Kim et al. (1987). Samples were randomly selected, thawed, and homogenized in 0.17 *M* perchloric acid (90 mg/ml) using a Polytron homogenizer (GLAS-COL, Terre Haute, IN) then centrifuged at 31,000 × g for 30 min at 4°C. The supernatants were separated and analyzed for concentrations of norepinephrine (NE), epinephrine (E), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA). Blood was homogenized with an equal volume of 0.34 *M* perchloric acid, and similarly processed. High-performance liquid chromatography (HPLC) determinations were performed with a Dionex model DX-300 isocratic liquid chromatography unit (Dionex Corp., Sunnyvale, CA) coupled with a pulse electrochemical detector (PED-2). A glassy-carbon working electrode was set at 0.8 V versus an Ag/AgCl reference electrode. The sensitivity of the detector was maintained between 0.5 and 1.0 nA, depending upon the concentration of the neurotransmitters. Separation by isocratic elution was performed on a C18 reverse-phase column preceded by a guard column (Guard-Pak, C18, Waters Association, Milford, MA). The mobile phase was 15% (v/v) methanol in solution of 32 m*M* citric acid (pH 4.2), 12.5 m*M* disodium hydrogen orthophosphate, 0.5 m*M* octyl sodium phosphate, and 0.05 m*M* ethylenediamine tetraacetic acid (EDTA). The mobile phase

ing a single testing period. Sessions during which the rat received only a series of eight 105-dB tones constituted the acoustic startle response (ASR) trials. Sessions during which rats received a series of eight 105-dB tones preceded (100 ms) by a 74-dB tone were the prepulse inhibition (PPI) trials. A single session containing eight presentations of a 74 dB tone was used as a baseline control. Total length of the eight auditory startle sessions was approximately 23 min; a 60-s adaptation period preceded testing, individual sessions were separated by 60-s intersession intervals, and individual pulses within sessions were separated by 10-s intertrial intervals. The dependent variable for each trial was amplitude of the startle response, as measured in newtons. The order of presentation of the eight sessions was as follows:

Session 1. ASR-1, 105-dB single startle pulse.
Session 2. Control, 74-dB single startle pulse.
Session 3. PPI-1, 74-dB prepulse paired with 105-dB startle pulse.
Session 4. ASR-2, 105-dB single startle pulse.
Session 5. PPI-2, 74-dB prepulse paired with 105-dB startle pulse.
Session 6. ASR-3, 105-dB single startle pulse.
Session 7. PPI-3, 74-dB prepulse paired with 105-dB startle pulse.
Session 8. ASR-4, 105-dB single startle pulse.

Test 7. Tail Flick Response (TFR) Rats were individually evaluated for integrity of analgesia systems using the tail-flick analgesia meter with 250–500 g rat restrainer (model 1420-D-30w/1434-2-D-30, Columbus Instruments, Columbus, OH). Each rat was placed in a clear Plexiglas rat restrainer tube such that the protruding posterior two-thirds of the tail was across the sensing slot of the apparatus. At this point, a manual switch was activated, initiating a timer and opening a shutter, exposing the tail to heat from a bulb located under the apparatus stage. In response to flicking of the tail, the timer was stopped and the shutter closed. The dependent measure was latency (seconds) from the opening of the shutter to activation of the photobeam. Each rat was tested for 3 trials separated by an ITI of approximately 10 s. The apparatus was operated on heat setting 12, which was measured at 37.8 ± 1.1°C (at 22 ± 1.8°C ambient room temperature) at the maximum 5 s of tail exposure. The dependent measure was the mean latency to tail flick for the three trials.

Test 8. Treadmill Physical Fatigue (TPF) Rats were evaluated for exercise fatigue utilizing a two-lane rat exerciser treadmill unit (model 0931-D-30, Columbus Instruments, Columbus, OH). The two-lane treadmill unit featured troughs at the end of each running lane, allowing delivery of negative reinforcement. Animals were initially pretrained on the apparatus for 5 min each at a forward lane speed of 0.1 m/s with an inclined lane angle of 15°, a speed and inclination angle at which all tested animals could easily avoided negative reinforcement. Negative reinforce-

mals for both the SRG and LRG conditions. Results of the blood serum assays for 5-HT and 5-HIAA levels are shown in Figure 1.

Regional Brain Neurotransmitter Assays

The brain neurotransmitter assays indicated a significantly greater concentration of 5-HT in the cerebellar, brainstem, hippocampal, and striatal brain regions of the V exposed group as compared to the AC group for the SRG condition, and a significantly greater concentration of 5-HT in the cerebellar, brainstem, cortical, and hippocampal brain regions of the V exposed group as compared to the AC group for the LRG condition. Similarly, 5-HIAA concentration was significantly greater in the cerebellar, brainstem, cortical, and hippocampal brain regions of the V exposed group as compared to the AC group for the SRG condition, and was significantly greater in the cerebellar, brainstem, cortical, and striatal brain regions of the V exposed group for the LRG condition. Results of the regional neurotransmitter assays for 5-HT and 5-HIAA levels are shown in Figure 2 (page 485).

Neurobehavioral Battery

Results of the eight NTAB tests utilized in this study are summarized in Figure 3.

Test 1. Forelimb Grip Strength (FGS) There was no significant difference between AC and V exposed rats in either the SRG or LRG condition.

Test 2. Photosensitivity (PS) There was no significant difference between AC and V exposed rats in either the SRG or LRG condition.

Test 3. Appetitive Reinforcer Approach Sensitization (ARAS) The V exposed rats in the SRG approached the baited stimulus areas significantly more than AC rats, spending nearly 300% more time than AC exposed rats approaching the raw hamburger meat stimuli. In the LRG condition there was no significant difference in approach times between AC and V exposed groups.

Test 4. Total Locomotor Activity (TLA) In the LRG condition, V exposed rats initiated significantly fewer horizontal beam breaks than did AC exposed rats, although there was no significant difference between means for the vertical beam breaks measure. In the SRG condition, there were no statistical differences between means for horizontal or vertical beam breaks between the AC and V exposed groups.

Test 5–6. Acoustic Startle Response–Prepulse Inhibition (ASR/PPI) For the acoustic startle response (ASR), there was no significant difference between AC and V exposed rats in either the SRG or LRG condition. In terms of prepulse inhibition (PPI of ASR), the V exposed rats in the LRG condition exhibited significantly decreased PPI of ASR compared to the AC exposed rats. In the SRG condition, there was no significant difference between V exposed and AC rats.

was filtered through a 0.45-mm filter (Millipore, Bedford, MA) and degassed under a vacuum before use. A flow rate of 1.2 ml/min (2200 pounds/in^2) at ambient temperature was employed. Known amounts of NE, E, DA, DOPAC, HVA, 5-HT, and 5-HIAA in the range 0.2–20 ng were injected into the HPLC system. The internal standard was 3,4-dihydroxybenzylamine hydrobromide (DHBA, 2.5 ng). All compounds were easily oxidized at 0.8 V versus an Ag/AgCl reference electrode. Each compound gave a linear response in the 0.2–20 ng range.

Statistics

All statistical analyses were conducted using GB STAT software (Dynamic Microsystems, Inc., Silver Springs, MD). Differences between the means for NTAB test results and for brain/blood neurotransmitter assays were analyzed utilizing the Student's *t*-test, or one-way analysis of variance (ANOVA) statistic, Levene's test of homogeneity, and post hoc Fisher's LSD (protected *t*-test). Differences between means were considered significant when $p \leq .05$.

RESULTS

Body Weights, Gross Examinations, and Tissue Histopathology

Throughout the course of the study there were no significant differences in body weights between AC and V group rats, based on comparison of the weekly mean weights of individual animals. No rats exhibited signs of physiological irritancy (i.e., respiratory distress, eye area inflammation, fur discoloration, or behavioral signs of pain and distress), and no animals died or were removed from the study. Gross examination of sacrificed rats indicated no observable differences between AC and V exposed rats, as measured by absolute organ weights and organ-to-body weight ratios. Examination of heart, lungs, liver, kidneys, spleen, stomach, duodenum, jejunum, ileum, colon, testes, thymus, femur bone marrow, sciatic nerve, skeletal muscle, skin, cervical lymph nodes, thyroids, parathyroids, adrenals, pituitary, and cervical and lumbar vertebrae with spinal cord tissue samples indicated no unusual pathology as compared to the appropriate AC group. Results of the blood hematology tests indicated no significant differences between AC and V exposure groups for either the SRG or LRG conditions. These results are available in an Air Force Technical Report (MacMahon et al., 1998).

Blood Serum Neurotransmitter Assays

There was a significantly higher concentration of 5-HT in the blood serum samples of V exposed rats compared to AC exposed animals for the SRG condition, but no marked difference between groups for the LRG condition. There were significantly higher concentrations of 5-HIAA in the blood serum samples of V exposed rats compared to AC exposed ani-

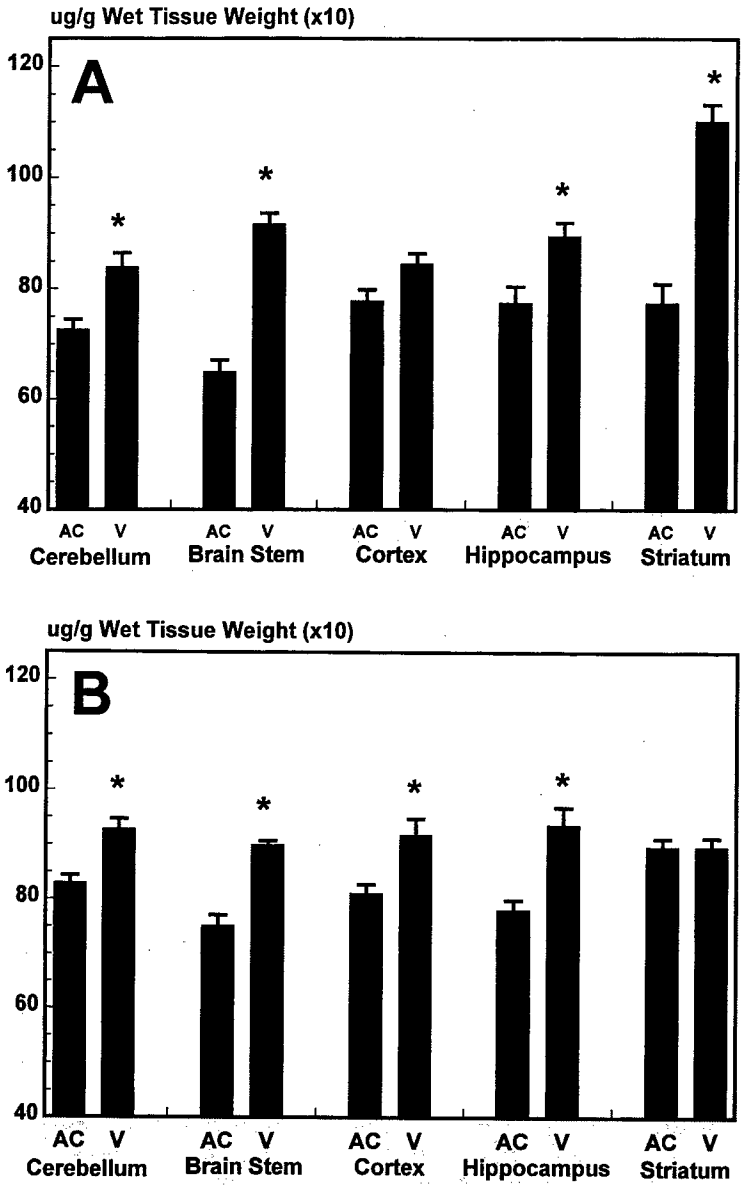


FIGURE 2. Mean concentrations of 5-hydroxytryptamine (5-HT) or 5-hydroxyindoleacetic acid (5-HIAA) in five brain regions of air control (AC) and JP-4 jet fuel vapor (V) exposed rats for the short recovery group (SRG) and long recovery group (LRG) conditions: (A) 5-HT for SRG; (B) 5-HT for LRG; All data are presented as mean + SEM; asterisk indicates $p \leq .05$ versus appropriate control.

DISCUSSION AND CONCLUSIONS

Exposure of Sprague-Dawley rats to JP-4 jet fuel vapor (2 mg/L ± 10%) for 6 h/d for 14 d resulted in time-dependent, postexposure differences between fuel-exposed and air control rats on several NTAB tests that were measured during a period in which 5-HT/5-HIAA levels were significantly

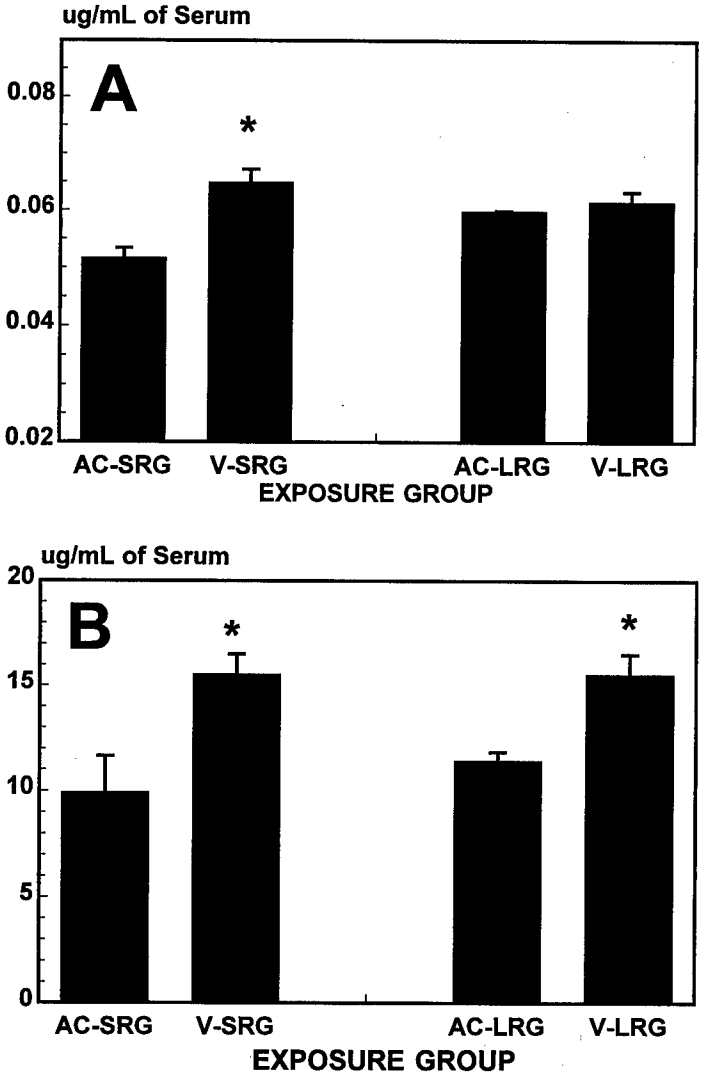


FIGURE 1. Mean concentrations of (A) 5-hydroxytryptamine (5-HT) or (B) 5-hydroxyindoleacetic acid (5-HIAA) measured in the blood serum of air control (AC) and JP-4 jet fuel vapor (V) exposed rats for the short recovery group (SRG) and long recovery group (LRG) conditions. All data are presented as mean + SEM; asterisk indicates $p \leq .05$ versus respective control

Test 7. Tail Flick Response (TFR) In the LRG condition, V exposed rats exhibited tail flicks that were significantly more rapid those observed from AC exposed rats, although in the SRG condition there was no significant difference between groups.

Test 8. Treadmill Physical Fatigue (TPF) There was no significant difference between AC and V exposed rats in either the SRG or LRG condition.

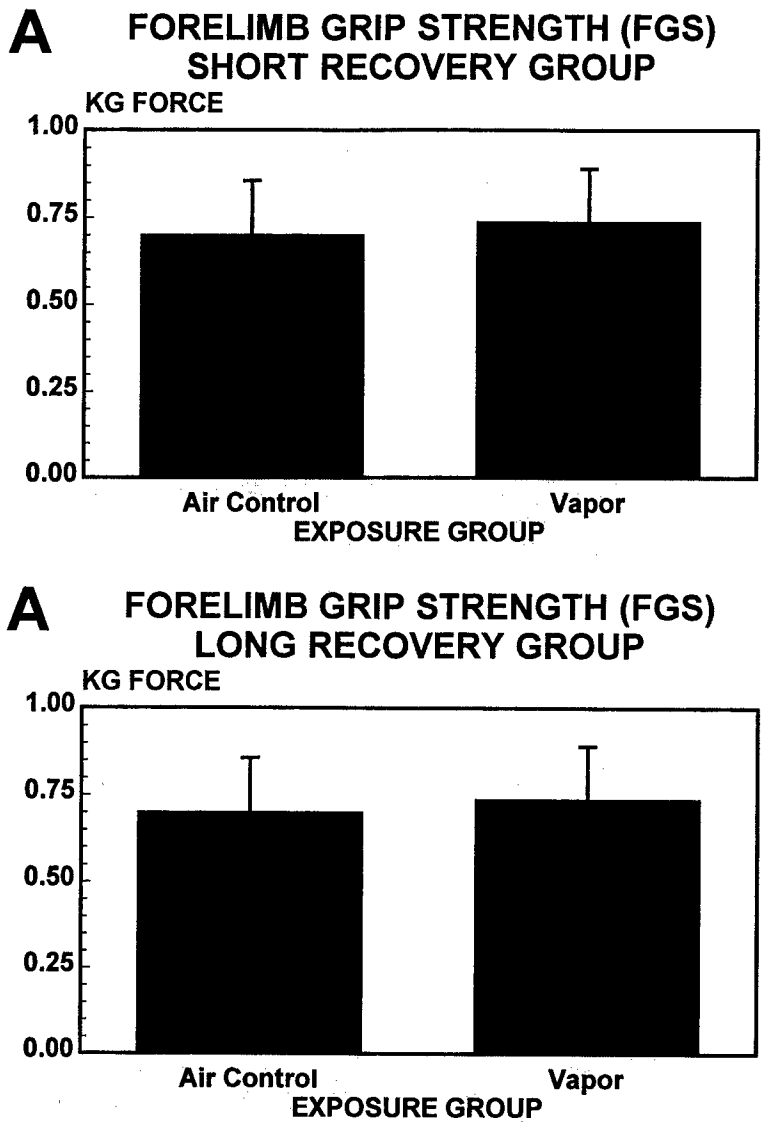


FIGURE 3. Performances of air control (AC) and JP-4 jet fuel vapor (V) exposed rats on eight NTAB tests for the short recovery group (SRG) or long recovery (LRG) group condition: (A) forelimb grip strength test. All data are presented as mean + SEM; asterisk indicates $p \leq .05$ versus respective control.

(Knave et al., 1976, 1978, 1979; Struwe & Wennberg, 1983; Struwe et al., 1983).

Among V exposed rats tested following a 14-d recovery (SRG), measured neurobehavioral changes were limited to significantly increased approach to a novel appetitive stimulus in the ARAS test, consistent with significant increases in cerebellar, brainstem, hippocampal, and striatal

increased in fuel-exposed subjects. In the absence of obvious signs of physiological irritancy, or significant changes in body weight, major organ weights and gross organ pathology, or blood hematology, these changes were subtle, requiring evaluation with a neurobehavioral battery, and to some degree modeled the previously described neurobehavioral changes in some humans exposed to hydrocarbon fuel vapor for from 4 to 32 yr

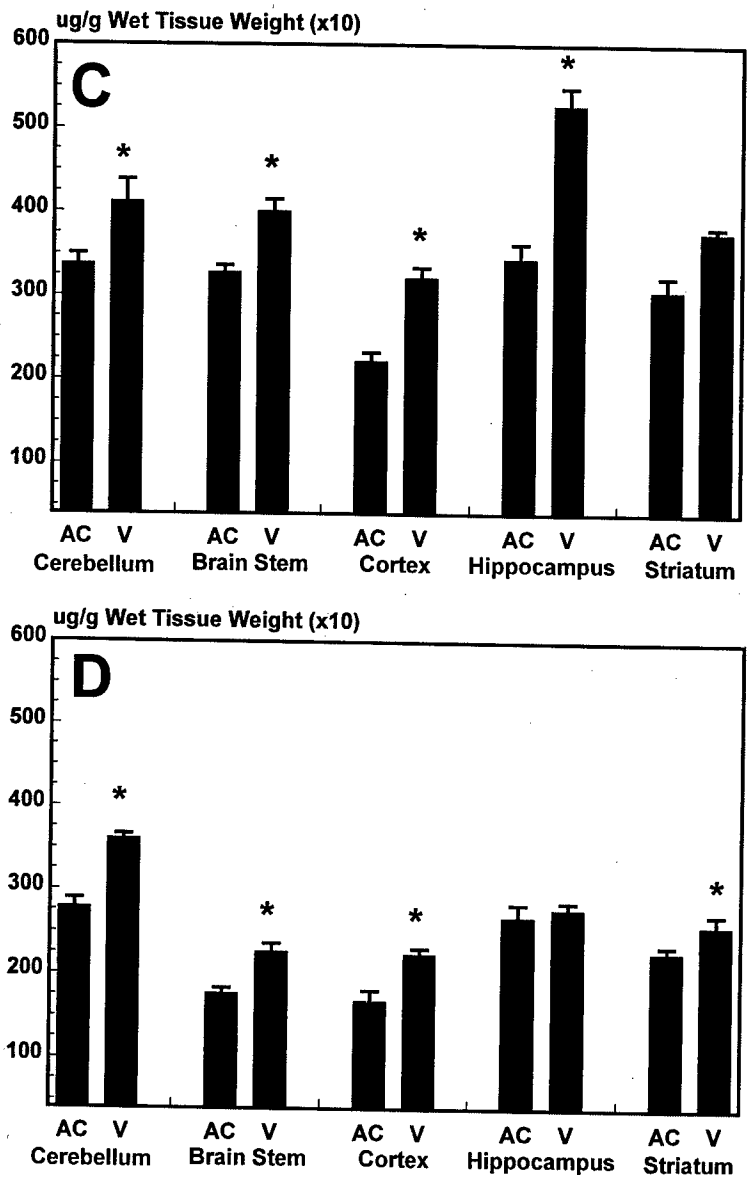


FIGURE 2. (*Continued*) Mean concentrations of 5-hydroxytryptamine (5-HT) or 5-hydroxyindoleacetic acid (5-HIAA) in five brain regions of air control (AC) and JP-4 jet fuel vapor (V) exposed rats for the short recovery group (SRG) and long recovery group (LRG) conditions: (C) 5-HIAA for SRG; (D) 5-HIAA for LRG. All data are presented as mean + SEM; asterisk indicates $p \leq .05$ versus appropriate control.

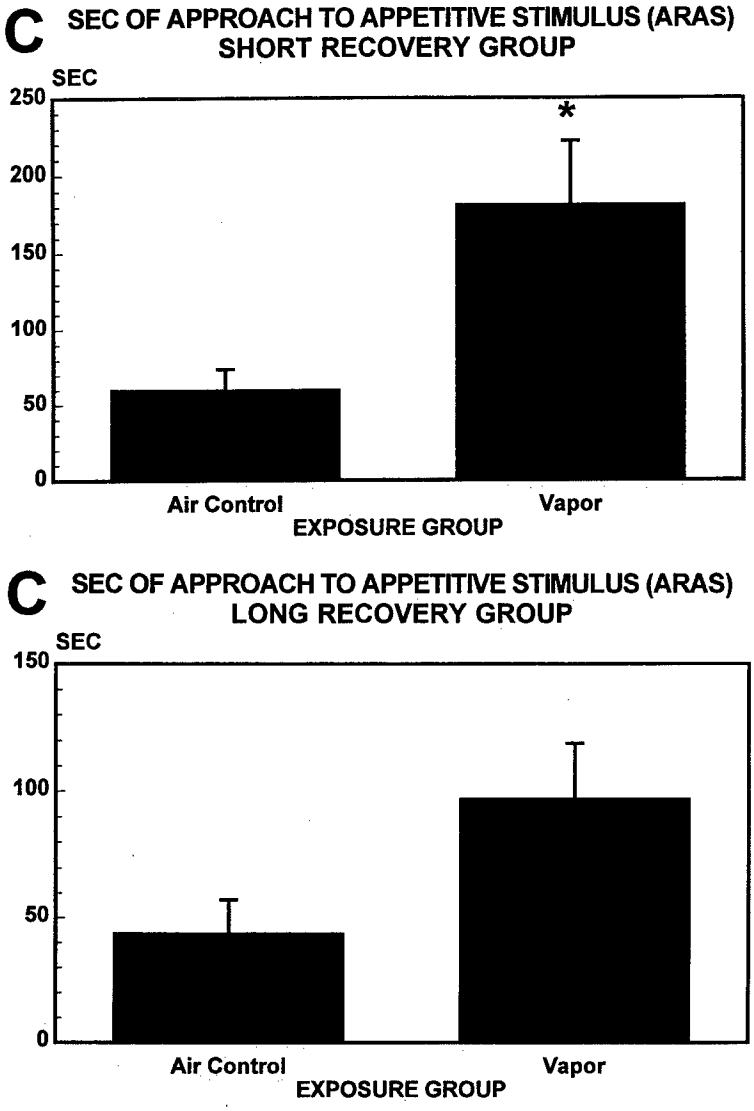


FIGURE 3. (*Continued*) Performances of air control (AC) and JP-4 jet fuel vapor (V) exposed rats on eight NTAB tests for the short recovery group (SRG) or long recovery (LRG) group condition: (C) ARAS test. All data are presented as mean + SEM; asterisk indicates $p \leq .05$ versus respective control.

an increase in 5-HT was unpredicted by the neurotransmitter analysis data. The more traditional amphetamine challenge test of dopamine circuit sensitization (as measured by changes in locomotor activity) could not be used due to possible biasing of subsequent neurobehavioral and neurotransmitter assay tests. It should noted, however, that the sacrifice of SRG animals occurred at 21–22 d postexposure (15–16 d following completion of the ARAS test) and, as previously reported (Bonhomme et

region 5-HT levels, cerebellar, brainstem, cortical, and hippocampal 5-HIAA levels, and significantly increased blood serum levels of 5-HT and 5-HIAA as compared to the appropriate AC group. As the ARAS test has been previously utilized to identify sensitization of dopaminergic circuits (Rossi III et al., 1996), this change in behavioral capacity consistent with

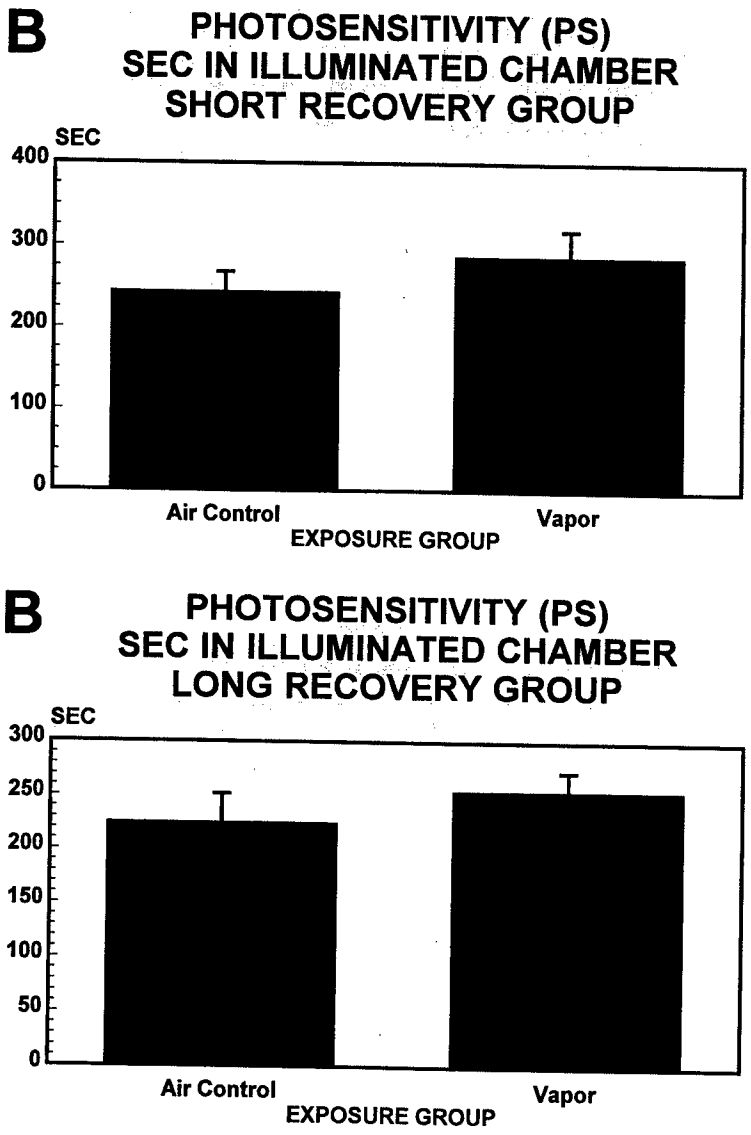


FIGURE 3. (*Continued*) Performances of air control (AC) and JP-4 jet fuel vapor (V) exposed rats on eight NTAB tests for the short recovery group (SRG) or long recovery (LRG) group condition: (B) photosensitivity test. All data are presented as mean + SEM; asterisk indicates $p \leq .05$ versus respective control.

al., 1995; De Deurwaerdere et al., 1996), dopamine (DA) and serotonin (5-HT) systems may be differently and time-dependently involved in behavioral sensitization. It is, then, possible that a transient, exposure-induced modulation of the DA system occurred during the time period between the beginning of the vapor exposures and the sacrifice of SRG condition subjects.

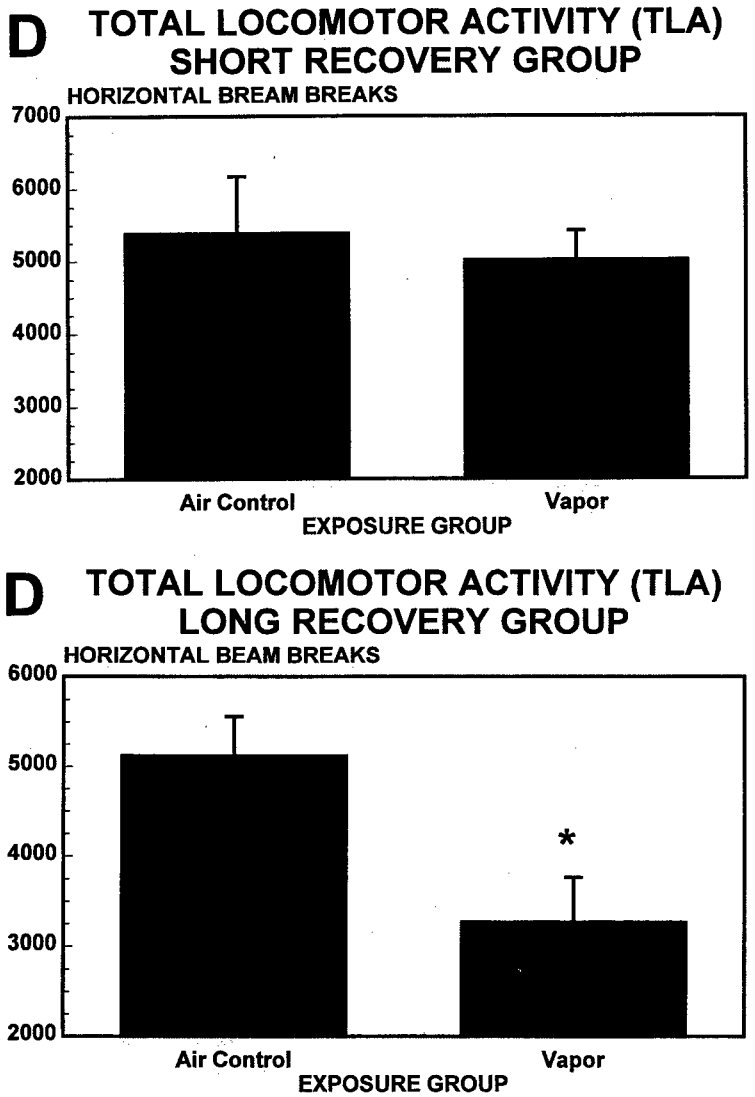


FIGURE 3. (*Continued*) Performances of air control (AC) and JP-4 jet fuel vapor (V) exposed rats on eight NTAB tests for the short recovery group (SRG) or long recovery (LRG) group condition: (D) total locomotor activity test. All data are presented as mean + SEM; asterisk indicates $p \leq .05$ versus respective control.

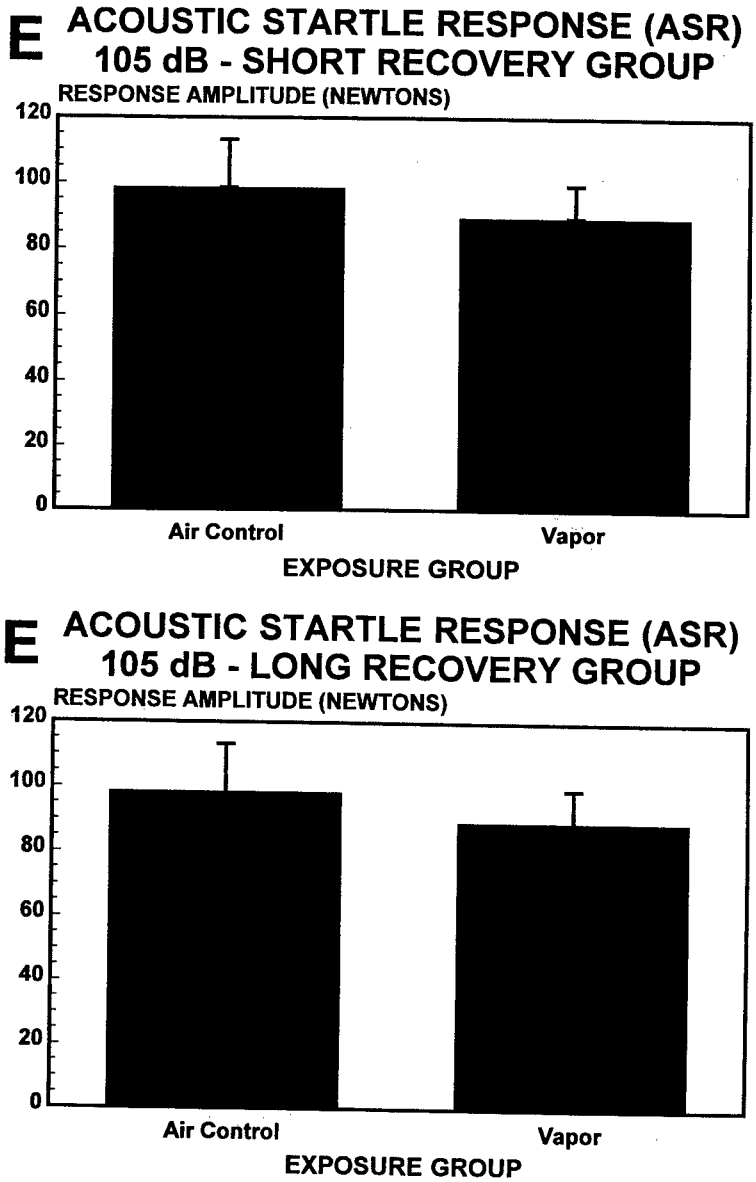


FIGURE 3. (*Continued*) Performances of air control (AC) and JP-4 jet fuel vapor (V) exposed rats on eight NTAB tests for the short recovery group (SRG) or long recovery (LRG) group condition: (E) acoustic startle response test. All data are presented as mean + SEM; asterisk indicates $p \leq .05$ versus respective control.

When evaluated for neurobehavioral capacity, rats rested for 60 d (LRG) exhibited a large numerical increase in seconds of approach to the appetitive stimulus (ARAS) combined with a significant decrease in horizontal beam breaks in the total locomotor activity (TLA) test, a significant decrease in prepulse inhibition of the acoustic startle response

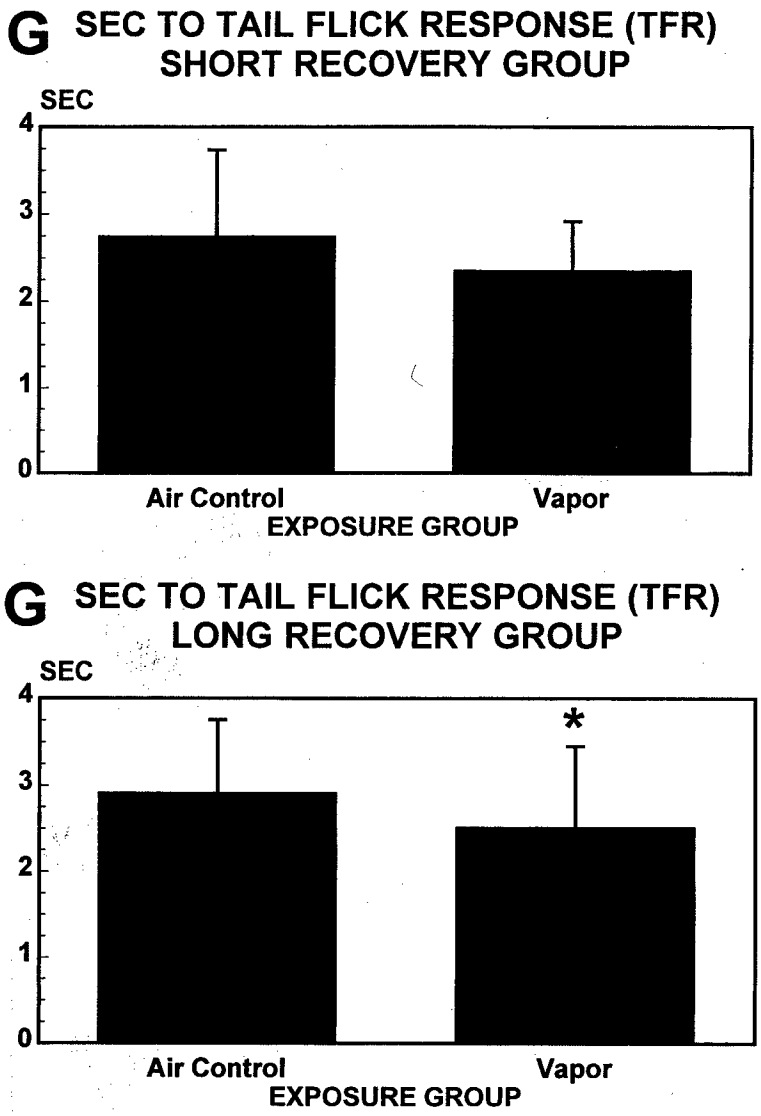


FIGURE 3. (*Continued*) Performances of air control (AC) and JP-4 jet fuel vapor (V) exposed rats on eight NTAB tests for the short recovery group (SRG) or long recovery (LRG) group condition: (G) tail flick response test. All data are presented as mean + SEM; asterisk indicates $p \leq .05$ versus respective control.

pared to the appropriate AC group. V exposed rats in the LRG condition (evaluated for changes in neurobehavioral capacity from 60 to 81 d post-exposure), then, exhibited a number of significant deficits as compared to AC exposed rats that were not detected in the identically exposed SRG condition. This implies that specific neurobehavioral effects of the JP-4 vapor exposures were time dependent and may have initially occurred as long as 60 d postexposure.

(PPI or ASR), and a significantly decreased response time on the tail flick response (TFR) test as compared to appropriate AC exposed rats. These neurobehavioral changes were consistent with significant increases in cerebellar, brainstem, cortical, and hippocampal region 5-HT levels, cerebellar, brainstem, cortical, and striatal 5-HIAA levels, and a significantly increased blood serum levels of 5-HIAA (but not 5-HT) as com-

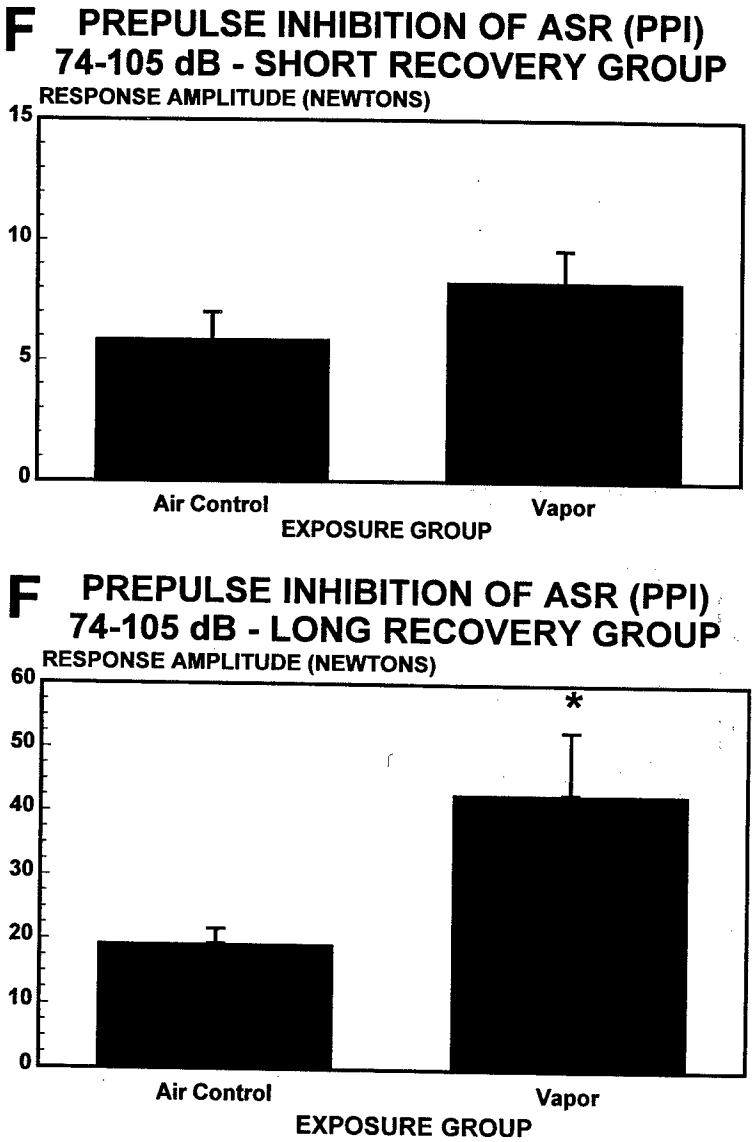


FIGURE 3. (*Continued*) Performances of air control (AC) and JP-4 jet fuel vapor (V) exposed rats on eight NTAB tests for the short recovery group (SRG) or long recovery (LRG) group condition: (F) prepulse inhibition of ASR test. All data are presented as mean + SEM; asterisk indicates $p \leq .05$ versus respective control.

modulation (particularly involving the 5-HT_{1A} and 5-HT_{2A} receptors) of the serotonergic or, in some cases, dopaminergic systems (i.e., nucleus accumbens overactivity) can decrease PPI of ASR in rats. Rigdon and Weatherspoon (1992), for example, have hypothesized that reduced PPI of ASR in rats resulting from administration of the 5-HT_{1A} receptor agonist 8-hydroxy-di-*n*-propylaminotetralin (8-OH-DPAT) may occur via dopaminergic mechanisms. Deficits measured in the LRG condition, as with the neurobehavioral deficit observed in the SRG condition, may have been partially induced by a transient modulation of the dopaminergic system possibly occurring between the V exposures and the measurement of the neurobehavioral deficits and increased 5-HT/5-HIAA levels.

The significantly increased tail flick response (TFR) response observed in LRG rats exposed to JP-4 vapor may, again, reflect exposure-induced modulation of the serotonin and/or dopamine systems. Several previously published reports (Alhaider & Wilcox, 1993; Bervoets et al., 1993) indicate that tail flick nociception is facilitated by agonistic modulation of supraspinal 5-HT_{1A} receptors and is reduced by antagonism of this system.

Finally, the decreased locomotor activity observed among V exposed rats in the LRG, but not in the SRG condition, is more difficult to explain with a serotonin/dopamine modulation hypothesis than are the other neurobehavioral changes. Among V exposed rats in the SRG condition, in which regional brain 5-HT and/or 5-HIAA levels were generally significantly increased, there was no difference in locomotor activity as compared to AC exposed rats. Among the LRG rats, in which regional 5-HT and 5-HIAA levels were also generally significantly greater than for AC exposed animals, horizontal beam breaks were significantly reduced, although vertical beam breaks and physical endurance (TLA test) measures were not different from those of appropriate control animals. It is unknown whether the differences in regional concentrations of 5-HT and 5-HIAA (i.e., for cortical, striatal, and hippocampal regions) detected between rats assayed following the SRG or LRG condition are related to the observed differences in neurobehavioral capacity. While there is abundant evidence that modulation of the serotonin system influences activity levels, specifically through interaction with DA (Chojnacka-Wojcik, 1992), cholinergic (Fujii et al., 1997), and noradrenergic systems (Nisijima & Ishiguro, 1995), there exists controversy about whether increased 5-HT levels or administration of 5-HT agonists increase or decrease locomotor activity in rats (Geyer, 1996). A possible explanation for the significantly decreased motor activity in the LGR but not the SRG conditions is a V-induced time-dependent sensitization of the DA or 5-HT systems. Sensitization of one or both of these receptor systems has previously been demonstrated following repeated exposure to psychostimulants, including amphetamine, metamphetamine, or cocaine (Robinson & Becker, 1986; Cunningham et al., 1992; Sorg et al., 1994; Kostrzewa et al., 1994; Kostrzewa, 1995; Geyer, 1996), and recently following expo-

Decreased PPI of ASR with no change in ASR is a commonly observed deficit in human nonparanoid schizophrenics and is thought to reflect reduced sensory gating, resulting from modulation of the 5-HT or DA systems (Geyer & Braff, 1987; Rigdon & Weatherspoon, 1992). As has been repeatedly shown (Swerdlow et al., 1990; Johansson et al., 1990; Varty & Higgins, 1995; Sipes & Geyer, 1995), overactivity or agonistic

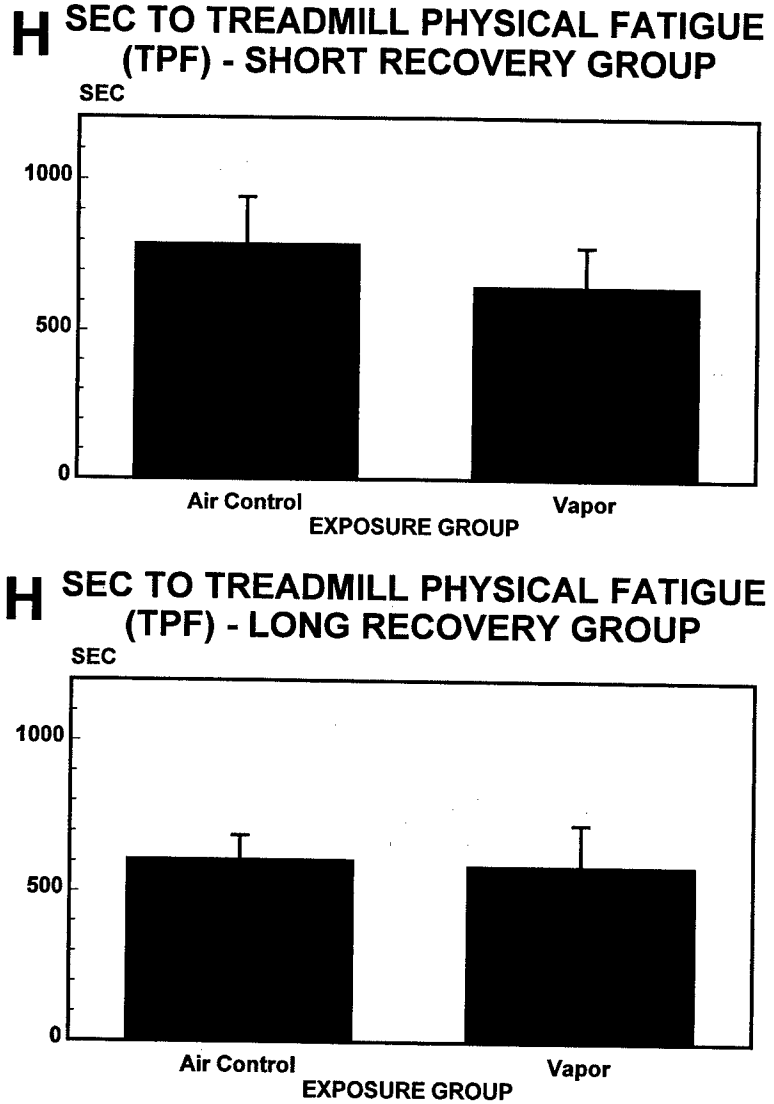


FIGURE 3. (*Continued*) Performances of air control (AC) and JP-4 jet fuel vapor (V) exposed rats on eight NTAB tests for the short recovery group (SRG) or long recovery (LRG) group condition: (H) treadmill physical fatigue test. All data are presented as mean + SEM; asterisk indicates $p \leq .05$ versus respective control.

Bell, I. R., Schwartz, G. E., Baldwin, C. M., Hardin, E. E., Klimas, N. G., Kline, J. P., Patarca, R., and Song, Z. Y. 1997b. Individual differences in neural sensitization and the role of context in illness from low-level environmental chemical exposures. *Environ. Health Perspect.* 105(Suppl. 2):457–466.

Bervoets, K., Rivet, J. M., and Millan, M. J. 1993. 5-HT1A receptors and the tail-flick response. IV. Spinally localized 5-HT1A receptors postsynaptic to serotoninergic neurons mediate spontaneous tail-flicks in the rat. *J. Pharmacol. Exp. Ther.* 264:95–104.

Benignus, V. A. 1981. Health effects of toluene: A review. *Neurotoxicology* 2:567–588.

Bonhomme, N., De Deurwaerdere, P., Le Moal, M., and Spampinato, U. 1995. Evidence for 5-HT4 receptor subtype involvement in the enhancement of striatal dopamine release induced by serotonin: A microdialysis study in the halothane-anesthetized rat. *Neuropharmacology* 34:269–279.

Bruner, R. H., Kinkead, E. R., O'Neill, T. P., Flemming, C. D., Mattie, D. R., Russell, C. A., and Wall, H. G. 1993. The toxicologic and oncogenic potential of JP-4 jet fuel vapor in rats and mice: 12-Month intermittent inhalation exposure. *Fundam. Appl. Toxicol.* 20:97–110.

Burbacher, T. M. 1993. Neurotoxic effects of gasoline and gasoline constituents. *Environ. Health Perspect.* 101(Suppl. 6):133–141.

Bushnell, P. J., Kelly, K. L., and Crofton, K. M. 1994. Effects of toluene inhalation on detection of auditory signals in rats. *Neurotoxicol. Teratol.* 16:149–160.

Chojnacka-Wojcik, E. 1992. Involvement of dopamine autoreceptors in the hypoactivity induced by 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) in mice. *Pol. J. Pharmacol. Pharm.* 44:135–146.

Contreras, C. M., Gonzalez-Estrada, T., Zarabozo, D., and Fernandez-Guardiola, A. 1979. Petit mal and grand mal seizures produced by toluene or benzene intoxication in the cat. *Electroencephalogr. Clin. Neurophysiol.* 46:290–300.

Cooper, J. R., and Mattie, D. R. 1996. Developmental toxicity of JP-8 jet fuel in the rat. *J. Appl. Toxicol.* 16:197–200.

Cunningham, K. A., Paris, J. M., and Goeders, N. 1992. Chronic cocaine enhances serotonin autoregulation and serotonin uptake binding. *Synapse* 11:112–123.

De Deurwaerdere, P., Bonhomme, N., Lucas, G., Le Moal, M., and Spampinato, U. 1996. Serotonin enhances striatal dopamine outflow in vivo through dopamine uptake sites. *J. Neurochem.* 66: 210–215.

Dossing, M., Loft, S., and Schroeder, E. 1985. Jet fuel and liver function. *Scand. J. Work Environ. Health* 11:433–437.

Fiedler, N., Kipen, H., Natelson, B., and Ottenweller, J. 1996. Chemical sensitivities and the Gulf War: Department of Veterans Affairs Research Center in basic and clinical science studies of environmental hazards. *Regul. Toxicol. Pharmacol.* 24:129–138.

Forkman, B. A., Ljungberg, T., Johnson, A. C., Nylen, P., Stahle, L., Hoglund, G., and Ungerstedt, U. 1991. Long-term effects of toluene inhalation on rat behavior. *Neurotoxicol. Teratol.* 13:475–481.

Fujii, T., Yoshizawa, M., Nakai, K., Fujimoto, K., Suzuki, T., and Kawashima, K. 1997. Demonstration of the facilitatory role of 8-OH-DPAT on cholinergic transmission in the rat hippocampus using in vivo microdialysis. *Brain Res.* 76:244–249.

Geyer, M. A. 1996. Serotonergic functions in arousal and motor activity. *Behav. Brain Res.* 73:31–35.

Geyer, M. A., and Braff, D. L. 1987. Startle habituation and sensorimotor gating in schizophrenia and related animal models. *Schizophr. Bull.* 13:643–668.

Gralewicz, S., Wiaderna, D., and Tomas, T. I. 1995. Development of spontaneous, age-related nonconvulsive seizure electrocortical activity and radial-maze learning after exposure to *m*-xylene in rats. *Int. J. Occup. Med. Environ. Health* 8:347–360.

Gralewicz, S., Wiaderna, D., Tomas, T., and Rydzynski, K. 1997. Behavioral changes following 4-week inhalation exposure to pseudocumene (1,2,4-trimethylbenzene) in the rat. *Neurotoxicol. Teratol.* 19:327–333.

Haley, R. W., Hom, J., Roland, P. S., Bryan, W. W., Van Ness, P. C., Bonte, F. J., Devous, M. D., Sr., Mathews, D., Fleckenstein, J. L., Wians, F. H., Jr., Wolfe, G. I., and Kurt, T. L. 1997a. Evaluation of neurologic function in Gulf War veterans. A blinded case-control study. *J. Am. Med. Assoc.* 277:223–230.

sure to formalin vapor (Sorg et al., 1996). Robinson and Becker (1986) concluded that behavioral sensitization or kindling is not unique to the psychopharmacology of stimulant drugs, but may be produced by many environmental stimuli that directly or indirectly activate brain catecholamine systems. A number of studies (Sorg et al., 1994; Bell et al., 1997a) have hypothesized that several human syndromes with poor medical diagnosis, including IEI and SBS, may involve chronic sensitization of catecholamine receptor systems resulting from repeated, low-level exposure to environmental chemical toxicants and stressors.

In conclusion, it was shown that repeated exposure of rats to JP-4 jet fuel vapor induced several changes in neurobehavioral capacity, that expression of these changes may depend upon length of the postexposure period, and that these changes may be temporally related to significant changes in brain and blood serum 5-HT/5-HIAA. As the present study utilized a battery of only eight performance tests, it must be assumed that evaluation with a more comprehensive neurobehavioral battery could identify additional changes. Because the vapor-exposed rats were evaluated for changes in neurobehavioral capacity at 14–35 or 60–81 d postexposure, it appears probable that evaluation at additional postexposure time points would reveal either additional changes in neurobehavioral capacity or changes in the magnitude of changes identified in this study. The significance of the increased levels of 5-HT/5-HIAA detected in the brains and serum of V exposed rats is yet undetermined but may warrant further study of serotonin/dopamine system sensitization. The neurobehavioral performance changes identified in rats exposed to JP-4 vapor in this study vary somewhat from those previously reported in human fuel or aircraft industry workers. It must, of course, be considered that the limited human exposure data available reflected effects of ongoing daily exposures from 4 to 32 yr to only estimated concentrations of a variety of jet fuel formulations and other VOCs, was largely based on self-reported symptoms, was limited to identification of subtle performance measured by the batteries of tests employed, and included only a limited battery of biomarkers. Likewise, it is presently unknown whether neurobehavioral consequences from exposure of rats to JP-4 jet fuel vapor can be generalized to similar exposures to other jet fuels. It appears, however, that the rodent model used in this study of JP-4 neurobehavioral toxicity holds promise for evaluating the acute and persisting effects of hydrocarbon fuel exposures.

REFERENCES

Alhaider, A. A., and Wilcox, G. L. 1993. Differential roles of 5-hydroxytryptamine 1A and 5-hydroxytryptamine 1B receptor subtypes in modulating spinal nociceptive transmission in mice. *J. Pharmacol. Exp. Ther.* 265:378–385.

Bell, I. R., Rossi, J. III, Gilbert, M. E., Kobal, G., Morrow, L. A., Newlin, D. B., Sorg, B. A., and Wood, R. 1997a. Testing the neural sensitization and kindling hypothesis for illness from low levels of environmental chemicals. *Environ. Health Perspect.* 105(Suppl. 2):539–547.

Miyagawa, M., Honma, T., and Sato, M. 1995. Effects of subchronic exposure to toluene on working and reference memory in rats. *Neurotoxicol. Teratol.* 17:657–664.

Niklasson, M., Stengard, K., and Tham, R. 1995. Are the effects of toluene on the vestibulo- and opto-ocular motor system inhibited by the action of $GABA_B$ antagonist CGP 35348. *Neurotoxicol. Teratol.* 17:351–371.

Nisijima, K., and Ishiguro, T. 1995. Cerebrospinal fluid levels of monoamine metabolites and gamma-aminobutyric acid in neuroleptic malignant syndrome. *J. Psychiatr. Res.* 29:233–244.

Odkvist, L. M., Arlinger, S. D., Edling, C., Larsby, B., and Bergholtz, L. M. 1987. Audiological and vestibulo-oculomotor findings in workers exposed to solvents and jet fuel. *Scand. Audiol.* 16:75–81.

Pfaff, J., Parton, K., Lantz, R. C., Chen, H., Hays, A. M., and Witten, M. L. 1995. Inhalation exposure to JP-8 jet fuel alters pulmonary function and substance P levels in Fischer-344 rats. *J. Appl. Toxicol.* 15:249–256.

Porter, H. O. 1990. Aviators intoxicated by inhalation of JP-5 fuel vapor. *Aviat. Space Environ. Med.* 61:654–656.

Pryor, G. T. 1991. A toluene-induced motor syndrome in rats resembling that seen in some human solvent abusers. *Neurotoxicol. Teratol.* 13:387–440.

Rahill, A. A., Weiss, B., Morrow, P. E., Frampton, M. W., Cox, C., Gibb, R., Gelein, R., Speers, D., and Utell, M. J. 1996. Human performance during exposure to toluene. *Aviat. Space Environ. Med.* 67:640–671.

Rigdon, C. C., and Weatherspoon, J. K. 1992. 5-Hydroxytryptamine 1a receptor agonists block prepulse inhibition of acoustic startle reflex. *J. Pharmacol. Exp. Ther.* 263:486–493.

Ritchie, G. D., Rossi, J. III, Macys, D. A., and Still, K. R. 1995. Application of the NMRI/TD Neurobehavioral Screening Battery to combustion toxicology. In *Fire and polymers II: Materials and tests for hazard prevention*, ed. G. Nelson, pp. 344–366. Washington, DC: ACS Books.

Robinson, T. E., and Becker, J. B. 1986. Enduring changes in brain and behavior produced by chronic amphetamine administration: A review and evaluation of animal models of amphetamine psychosis. *Brain Res.* 396:157–198.

Rossi, J. III, Bekkedal, M. Y., Knutson, B., Ritchie, G. D., and Panksepp, J. 1996. Long-term behavioral sensitization induced by a bridged organophosphate. *Soc. Neurosci. Abstr.* 22:2091

Sipes, T. A., and Geyer, M. A. 1995. 8-OH-DPAT disruption of prepulse inhibition in rats: Reversal with (+)WAY 100,135 and localization of site of action. *Psychopharmacol. Berl.* 117:41–81.

Smith, E. A., Kimmel, E. C., English, J. H., Bowen, L. E., Reboulet, J. E., and Carpenter, R. L. 1997. Evaluation of the respiratory tract after acute exposure to a pyrotechnically generated aerosol fire suppressant. *J. Appl. Toxicol.* 17:95–103.

Sorg, B. A., Hooks, M. S., and Kalivas, P. W. 1994. Neuroanatomy and neurochemical mechanisms of time-dependent sensitization. *Toxicol. Ind. Health* 10:369–386.

Sorg, B. A., Willis, J. R., Nowatka, T. C., Ulibarri, C., See, R. E., and Westberg, H. H. 1996. Proposed animal neurosensitization model for multiple chemical sensitivity in studies with formalin. *Toxicology* 111:135–145.

Struwe, G., Knave, B., and Mindus, P. S. 1983. Neuropsychiatric symptoms in workers occupationally exposed to jet fuel—A combined epidemiological and causistic study. *Acta Psychiatr. Scand.* 67 (Suppl. 303):55–67.

Struwe, G., and Wennberg, A. 1983. Psychiatric and neurological symptoms in workers occupationally exposed to organic solvents—Results of a differential epidemiological study. *Acta Psychiatr. Scand.* 67(Suppl. 303):68–80.

Swerdlow, N. R., Mansbach, R. S., Geyer, M. A., Pulvirenti, L., Koob, G. F. and Braff, D. L. 1990. Amphetamine disruption of prepulse inhibition of acoustic startle is reversed by depletion of mesolimbic dopamine. *Psychopharmacol. Berl.* 100:413–446.

van der Staay, F. J., and Blokland, A. 1996. Behavioral differences between outbred Wistar, inbred Fischer 344, Brown Norway, and hybrid Fischer 344 × Brown Norway rats. *Physiol. Behav.* 60:97–109.

Varty, G. B., and Higgins, G. A. 1995. Examination of drug-induced and isolation-induced disruptions of prepulse inhibition as models to screen antipsychotic drugs. *Psychopharmacol. Berl.* 122: 15–26.

Haley, R. W., Kurt, K. L., and Hom, J. 1997b. Is there a Gulf War Syndrome? Searching for syndromes by factor analysis of symptoms. *J. Am. Med. Assoc.* 277:215–222.

Harris, D. T., Sakiestewa, D., Robledo, R. F., and Witten, M. 1997. Immunotoxicological effects of JP-8 jet fuel exposure. *Toxicol. Ind. Health* 13:43–55.

Ikeda, T., Katakura, Y., Kishi, R., and Miyake, H. T. 1993. Acute neurobehavioral effects of co-inhalation of toluene and *n*-hexane on schedule-controlled behavior in rats. *Environ. Res.* 63: 70–81.

Jamal, G. A., Hansen, S., Apartopoulos, F., and Peden, A. 1996. The "Gulf War syndrome." Is there evidence of dysfunction in the nervous system? *J. Neurol. Neurosurg. Psychiatry* 60:449–451.

Johansson, C. E., Meyerson, B. J., and Hoglund, A. U. 1990. The long-term effects of 8-hydroxy-2-(di-*n*-propyl-amino) tetralin (8-OH-DPAT) on copulatory and exploratory behaviour in male rats. *Eur. J. Pharmacol.* 178:1–9.

Kim, C., Speisky, M. B., and Kharouba, S. N. 1987. Rapid and sensitive method for measuring norepinephrine, dopamine, 5-hydroxytryptamine and their major metabolites in rat brain by high-performance liquid chromatography. Differential effect of probenecid, haloperidol and yohimbine on the concentrations of biogenic amines and metabolites in various regions of rat brain. *J. Chromatogr.* 386:25–35.

Kinkead, E. R., Wolfe, R. E., Bunger, S. K., and Leahy, H. F. 1992. The acute toxicity evaluation of a low-temperature hydraulic fluid. *Am. Ind. Hyg. Assoc. J.* 53:163–168.

Knave, B., Persson, H. E., Goldberg, J. M., and Westerholm, P. S. 1976. Long-term exposure to jet fuel: An investigation on occupationally exposed workers with special reference to the nervous system. *Scand. J. Work Environ. Health* 2:152–164.

Knave, B., Olson, B. A., Elofsson, S., Gamberale, F., Isaksson, A., Mindus, P., Persson, H. E., Struwe, G., Wennberg, A., and Westerholm, P. S. 1978. Long-term exposure to jet fuel. II. A cross-sectional epidemiologic investigation on occupationally exposed industrial workers with special reference to the nervous system. *Scand. J. Work Environ. Health* 4:19–45.

Knave, B., Mindus, P., and Struwe, G. S. 1979. Neurasthenic symptoms in workers occupationally exposed to jet fuel. *Acta Psychiatr. Scand.* 60:39–49.

Kostrzewa, R. M. 1995. Dopamine receptor supersensitivity. *Neurosci. Biobehav. Rev.* 19:1–17.

Kostrzewa, R. M., Brus, R., Kalbfleisch, J. H., Perry, K. W., and Fuller, R. W. 1994. Proposed animal model of attention deficit hyperactivity disorder. *Brain Res. Bull.* 34:161–171.

Leahy, H. F. 1996. Overview of the vapor generation and analysis parameters of the petroleum- and shale-derived fuel studies conducted in Thomas dome exposure chambers at the Toxic Hazards Research Unit, Wright-Patterson Air Force Base (Dayton), Ohio, 1973–1983. In *Toxic Hazards Research Unit Annual Technical Report: Govt. Reports Announcements & Index (GRA&I),* eds. J. D. MacEwen and E. H. Vernot, AL/OE-TR-1996, Issue 05.

MacMahon, K. L., Eggers, J., Wolfe, R. E., Leahy, H. F., Narayanan, L., Rossi, J. III, Nordholm, A., Ritchie, G., and Witzmann, F. 1998. Evaluation of exposure to pyridostigmine bromide, diethyltoluamide, JP-4 jet fuel and stress on male Sprague-Dawley rats. *Toxic Hazards Research Unit Annual Technical Report: Govt. Reports Announcements & Index (GRA&I)* AL/OE-TR-1997, Issue 06.

Macys, D. A., Carpenter, R. L., Risher, J. F., Vinegar, A., Dodd, D. E., and Wall, H. G. 1992. Results of a Workshop on Health Effects of Crude Oil Exposures Related to Operation Desert Storm. Naval Medical Research Institute, Government Publication, NMRI 92-04.

Mattie, D. R., Alden, C. L., Newell, T. K., Gaworski, C. L., and Flemming, C. D. 1991. A 90-day continuous vapor inhalation toxicity study of JP-8 jet fuel followed by 20 or 21 months of recovery in Fischer 344 rats and C57BL/6 mice. *Toxicol. Pathol.* 19:77–87.

Mattie, D. R., Marit, G. B., Flemming, C. D., and Cooper, J. R. 1995. The effects of JP-8 jet fuel on male Sprague-Dawley rats after a 90-day exposure by oral gavage. *Toxicol. Ind. Health* 11:423–435.

Mattie, R. R., Marit, G. B., Flemming, C. D., Sterner, T. R., and Cooper, J. R. 1996. The effects of JP-8 jet fuel on female Sprague-Dawley rats after a 21-week exposure by oral gavage. *Toxicologist* 30:44.

Meyer, O. A., Tilson, H. A., Byrd, W. C., and Riley, M. T. 1979. A method for the routine assessment of fore- and hindlimb grip strength of rats and mice. *Neurobehav. Toxicol.* 1:233–236.

von Euler, G., Ogren, S. O., Li, X. M., Fuxe, K., and Gustafsson, J. A. 1993. Persistent effects of subchronic toluene exposure on spatial learning and memory, dopamine-mediated locomotor activity and dopamine D2 agonist binding in the rat. *Toxicology* 77:223–232.

Wolfe, R. E., Kinead, E. R., Feldmann, M. L., Leahy, H. F., and Jederberg, W. W. 1997. Acute toxicity evaluation of JP-8 Jet Fuel and JP-8 Jet Fuel containing additives. *Govt. Reports Announcements & Index (GRA&I)*, Issue 09.

THE NEUROBEHAVIORAL TOXICITY OF JP-8, JP-5 AND JP-4 JET FUELS*

LT A.F. Nordholm, MSC, USNR, [1]G.D. Ritchie, [2]W. Malcolm, R.L. Carpenter,
CDR John Rossi III, MSC, USN and CAPT K.R. Still, MSC, USN
Naval Health Research Center Detachment-Toxicology (NHRC-TD),
[1]Geo-Centers, Inc., and [2]ManTech Environmental Services, 2612 Fifth St.,
Building 433, Wright-Patterson Air Force Base (WPAFB)
Dayton, OH 45433 USA

ABSTRACT (U)

(U) Groups of Sprague-Dawley rats (n = 32) were exposed by whole body inhalation methods to JP-8 (1.0 mg/L±10%) or JP-5 (1.2 mg/L±10%) vapor for 6 hr/day, 5 days/wk, for 6 wk; to JP-4 (2.0 mg/L±10%; n = 16) vapor for 6 hr/day for 14 consecutive days; or to appropriate room air control conditions. The concentrations were selected to represent real world vapor exposures that might be experienced by the operational military. Following conclusion of the exposures, rats were rested for 14-65 days, then were evaluated for persisting neurobehavioral deficits with a battery of tests selected from the Neurobehavioral Toxicity Assessment Battery (NTAB) for their presumed ability to identify performance deficits similar to those previously identified in chronically exposed European jet engine workers. NTAB tests utilized included: Forelimb Grip Strength (FGS), evaluating muscle strength; General Locomotor Activity (GLA); Forced Treadmill (FT), measuring physical fatigue; Tail Flick Response (TFR), evaluating nociception; Acoustic Startle Response/Prepulse Inhibition (ASR/PPI), quantifying auditory brainstem function and inhibition; Passive Avoidance (PA), evaluating short-term memory; Porsolt Forced Swim test (PFST), measuring emotional depression; Morris Water Maze (MWM), quantifying spatial localization and short term memory; Appetitive Reinforcer Approach Sensitization (ARAS), evaluating central nervous system (CNS) sensitization and dopaminergic function; and Conspecific Approach (CA), evaluating social behavior. Following sacrifice, regional CNS tissues were evaluated for changes, from air controls, in levels of neurotransmitters and major metabolites.

INTRODUCTION (U)

JET FUEL TOXICITY (U)

(U) Several billion gallons of jet fuel are manufactured and consumed internationally each year, with an estimated US military tri-service utilization of approximately 2.2 billion gallons in 1996. Jet fuels are petroleum middle distillate (PMD) fuels, containing from 150-400 long- and short-chain aliphatic and aromatic hydrocarbon compounds (C4-C17) that distill between 170-370° C. Jet fuels vary in chemical composition as a function of crude oil stock, refining methods, fuel type, manufacturer, lot, and additive performance packages. Jet fuels consist of varying concentrations of alkanes (n-paraffins & naphthas), branched alkanes (isoparaffins), cycloalkanes, alkenes (olefins), alkylbenzenes, dicycloalkanes, dicycloparaffins, indans, tetralines, polynuclear aromatic hydrocarbons (PAHs), and aromatics, in addition to chemical blending agents (ethers and alcohols) and additives that reduce

---

*This work was funded by the US Army Medical Research and Materiel Command (USAMRMC).

Opinions expressed in this manuscript are those of the authors and do not necessarily represent the positions of the Naval service, the US Army, or of the US military service at-large.

JP-4 exposure results reported previously in: Nordholm, A.F., Rossi III, J., et al. 1999. Repeated exposure of rats to JP-4 vapor induces changes in neurobehavioral capacity and 5HT/5-HIAA levels. *J. Toxicol. Environ. Health* 56:471-499.

Enclosure (2)

oxidation, deactivate metals, limit icing, increase lubricity, reduce flammability and electrical conductivity, or increase thermal stability properties. Several different jet fuel formulations have been utilized in the military (jet propellants JP-4, JP-5, JP-7, JP-8, JP-8+100, JP-TS, etc.), each containing varying concentrations of a number of short-chain aromatic hydrocarbon compounds with potential for human toxicity in general, and neurotoxicity in particular. Jet fuels include from 2-25% (by volume) concentrations of the aromatic hydrocarbons, benzene, toluene, *m-*, *o-*, *p-*xylene, and trimethylbenzene, in addition to potentially neurotoxic naphthas and the light alkane *n*-hexane. Detailed discussions of military jet fuels and fuel-induced neurotoxicity can be located in a number of journal articles and government reports (Kinkead, 1974; Spencer, 1984; Gaworski et al., 1984; Clark et al., 1989; Shalka, 1989; Vernot et al., 1990; Seldon and Ahlborg, 1991; Macys et al., 1992; ATSDR, 1993; COT, 1996; Wolfe et al., 1997; Bakshi and Henderson, 1998). Exposure limits for jet fuels presently vary substantially, depending upon issuing agency or military service, ranging generally from a PEL (Permissible Exposure Limit in air averaged over 8-hr) of 350 ppm to a STEL (Short Term Exposure Limit, or maximum concentration allowable for 15 min exposure, no more than 4 times/day) of 1800 ppm.

MILITARY JET FUEL APPLICATIONS (U)

(U) Before 1991, there was nearly exclusive use of JP-4 jet fuel by the US Air Force (USAF) and US Army for the powering of jet aircraft and helicopters. Since 1991, there has been a transition by the USAF and US Army to nearly exclusive reliance upon JP-8 jet fuel for aircraft applications. The US Navy/Marines Corps has utilized JP-5 jet fuel for all aircraft applications for over 20 years, related to its lower shipboard flammability potential than JP-4 or JP-8, but is presently converting to use of JP-8 to accommodate the Joint Strike Fighter (JSF) on aircraft carriers. Jet fuels are additionally utilized by the military to power a wide range of land vehicles, as a heat sink (coolant) on jet aircraft, and for occasional misapplications including the powering of tent heaters and the suppression of desert silicon sand aerosols. Other hydrocarbon fuels, including diesel fuel (DF), diesel fuel marine (DFM), unleaded gasoline, and kerosene are also commonly used by the US military, and contribute to complex mixture exposure scenarios. While the militaries of the most North American Treaty Organization (NATO) allies are predominantly using JP-8 jet fuel for avionics applications, there was extensive use of "JP-4-like" fuels (e.g., MC-75 and MC-77) before 1991.

MILITARY EXPOSURE SCENARIOS (U)

(U) Military personnel may be exposed to raw jet fuels through dermal contact, inhalation or, in rare occasions, through oral ingestion related to the contamination of potable water or food sources. Inhalation of jet fuel vapors or aerosol, or the combustion byproducts of jet fuel occurs commonly during military deployment. Regardless of intended precautions, the detectable presence of jet fuel vapor/aerosol due to leakage, spillage, and normal venting during fuel transfer operations is extremely common. Occupational ratings in which jet fuel exposures most commonly occur include: fuel transportation and storage, fueling/defueling for ships, aircraft and land vehicles, fuel cell cleaning, maintenance and repair, bilge cleaning, jet engine maintenance and repair, and emergency response (spill clean-up). In the worst case scenario, measurements of jet fuel aerosol concentrations exceeding 15,000 mg/m^3 have been recorded in or near the openings to military fuel cells during cleaning operations. While chemically resistant eyewear, footwear and gloves may be worn by fuel handlers, there is no common use of respiratory protection or additional protective clothing to minimize exposures. While dermal absorption rates for jet fuels are generally unknown, it is considered that the most water soluble components of these hydrocarbon mixtures (i.e., benzene, toluene, xylene) account for the highest rates of dermal absorption. Contamination of water supplies used for showering aboard military ships and in combat or training theaters provides an additional source of dermal exposure to jet fuels. The US Navy has expressed increasing concern for the health of sailors exposed to possible combinations of JP-5, JP-8 and diesel fuel marine (DFM) during the servicing and fueling of tanks, fuel hauling vehicles, other trucks, jeeps, trailers, and helicopters stored in newly designed sealift ships.

NEUROBEHAVIORAL CONSEQUENCES OF HYDROCARBON FUEL EXPOSURE (U)

(U) The neurobehavioral consequences of acute exposure to gasoline or other hydrocarbon fuels have been reported in the literature beginning as early as 1872 (Kearney and Dunham, 1986; Page and Mehlman, 1989; Burbacher, 1993). Early testing of gasoline as an anesthetic resulted in reported sleep and anesthesia in volunteers

exposed to vapor from 20-40 gm gasoline over a period of 8-12 min. Studies have described children and young adults who have become chronic gasoline sniffers (Clinger and Johnson, 1951; Easson, 1962), with reported effects including: confusion, lack of self-control, excitement, combativeness, blurred vision, incoordination, depression, lethargy, headaches, roaring in the ears, trembling, nausea, chest oppression, respiratory distress, and staggering gait. Drinker et al. (1943) reported that brief exposure to 1000 ppm of automotive gasoline induced slight dizziness, nausea and headache in human volunteers, while exposure to 2,600 ppm caused "drunkenness" and partial anesthesia. More recently Maruff et al. (1998), evaluating chronic petrol-sniffers, measured higher rates of abnormal tandem gait, rapid alternating hand movements, finger to nose movements, postural tremor, bilateral palmomental reflexes and brisk deep reflexes. Cognitive deficits occurred in the areas of visual attention, visual recognition memory and visual paired associate learning.

Nuttall provided, in 1958, a 2-year report of the neurobehavioral consequences of USAF jet fuel exposures. Symptoms reported from such exposures commonly included: disturbance of consciousness, nausea, headache, visual disturbance, and irritation of the eyes and nose. Davies (1964) reported the neurobehavioral effects of the accidental exposure of a USAF pilot to JP-4 (estimated 3000-7000 ppm) jet fuel vapor/aerosol during a routine flight. During his emergency descent for landing, the pilot reported being lost, demonstrated an erratic landing roll, and did not follow the instructions of landing tower personnel. Similarly, Porter (1990) reported the case of intoxication of two military aviators by inhalation of JP-5 fuel vapors. One or both aviators experienced burning eyes, nausea, fatigue, impairment of eye-hand coordination, euphoria, and memory defects. During the emergency landing approach, a student aviator in the cockpit was reported to be laughing and euphoric. Porter further described acute effects of JP-5 exposure of students and pilots that included difficulty in recalling emergency procedures, flight plan information, and, in the extreme cases, personal information (i.e., wife's name). More recently Smith et al. (1997) utilized the postural stability technique to examine effects of exposure to jet fuels on 27 USAF workers (20 male and 7 female) with from 0.8-30 years (mean = 12.0 years) occupational exposure to jet fuels (mean years of exposure to JP-8 jet fuel = 4.56). The level of JP-8 exposure was measured by industrial hygiene techniques for two separate 8-hr periods for each exposed worker. Jet fuel exposures were expressed in several manners, including acute exposure based on 8-hr time weighted average (TWA). The TWAs for all exposed subjects were: benzene (5.90 ± 1.10 ppb), toluene (9.80 ± 2.60 ppb), *m-,o-,p*-xylene (7.50 ± 2.1 ppb), and naphthas (0.54 ± 0.12 ppm). Subjects were tested for postural sway with eyes open (EO) and eyes closed (EC), either standing on the bare surface of a postural balance apparatus or standing on a 4" thick section of foam. Smith et al. (1997) reported significant increases in postural body sway in the fuel-exposed group, most apparent in the eyes closed, standing of foam condition, as compared to non-exposed controls. When these deficits within the fuel exposed group were analyzed as a function of cumulative 8-hr exposures to the aromatic hydrocarbon constituents JP-8, there were significant correlation between the deficit and concentrations of benzene, toluene, and xylene, but not naphtha. The most significant relationship, however, occurred between postural sway length (eyes closed, and on 4" thick foam) and the average cumulative exposure to JP-8 benzene, and perhaps indicates subtle deficits in vestibular/proprioceptive systems. The results reported by Smith et al. (1997) are generally consistent with reports of polyneuropathy in jet-fuel exposed Scandinavian workers, as described below, when it is considered that both postural sway and limb vibratory threshold may be increased in subjects diagnosed with peripheral neuropathy (Bergin et al., 1995).

In these studies reported during the late 1970's and 1980's, (Knave et al., 1976, 1979; Mindus et al., 1978; Struwe et al., 1983a and 1983b; Holm et al., 1987) the neurobehavioral effects of acute and chronic exposure to jet fuels were evaluated in Scandinavian jet engine manufacturing and installation plant workers. In this plant, jet engines were both manufactured and tested, such that the majority of workers seldom or never experienced jet fuel, while employees in the fuel system manufacturing, installation and testing areas were repeatedly exposed. In the first study (Knave et al., 1976), based on personal interviews and company records, 29 workers were determined to be "considerably" exposed to jet fuel for a minimum of five years from 1960-1974, and without existence of chronic disease that might account for symptoms of neuroasthenia or polyneuropathy. Of these 29 workers, 13 were assigned to Group A, the "high" exposure group (exposed to high concentrations of jet fuel from a maximum of 1-2 hr/day, 5 days/wk, to a minimum of 20-30 min, once every three weeks) and the remaining 16 to Group B, the "lower" exposure group (20-30 min exposure for a maximum of once per month). In the worst case scenarios, workers spent approximately 50% of the work day 20-40 cm from objects perfused in jet fuel, or from 1-2 hr/day with the head and neck within a semi-enclosed tank containing residual jet fuel. In this study, estimates of approximate exposure concentrations were not provided, and no control group was evaluated. For chronic exposure to jet fuel, the following symptoms were self-reported or determined by medical examination for members of Group

A: distal paresthenia (77%); dizziness (77%); thoracic oppression (62%); depression/anxiety (62%); restless legs (62%); headache (31%); sleep disturbances (38%); respiratory symptoms (46%); muscle cramps (23%); irritability (23%); extremity pain (31%); memory impairment (15%); abnormal temperature sensibility (69%); abnormal pain sensibility (31%); abnormal tactile discrimination sensibility (15%); increased carpal vibratory threshold (neurological examination). Because these studies lumped fuel exposed workers into exposure groups, with minimal consideration of occupational and exposure history, age and education, and pre-existing medical conditions, and without use of matched control groups, the epidemiological interpretation of the results has been tenuous.

To establish the validity of the previously discussed study, Knave et al. (1978) conducted an additional study providing approximate jet fuel exposure concentrations and a comparison of results from 30 jet fuel exposed workers with data from 60 non-exposed controls. The 30 "exposed" workers were selected by a committee of consensus as being the "most heavily exposed", and consisted of 15 fuel system testers, 7 engine testers, and 8 mechanics (19 male workers and 11 male foremen) with an average of 17.1 years of occupational exposure (mean age = 46.4 years). Initially, 30 workers from the same plant were matched for "general health status", age, and duration of employment. A second control group was subsequently selected and matched for age, education, duration of employment, and trade union involvement. Quantitative air sampling studies indicated that fuel-exposed personnel were routinely exposed to an average of from 85-974 mg/m^3 (< 1 mg/L) with a range of from 41-3,226 mg/m^3, for a mean time of from 10-97 min per operation. The fuels used for testing in this plant were Swedish Armed Forces jet fuels MC-75 and MC-77, combinations of kerosene and unleaded gasoline, containing the aromatic hydrocarbons toluene, benzene, n-hexane, xylene, and trimethylbenzene at $\leq$ 1% (weight/weight) each. When compared to the matched controls, fuel-exposed workers reported or exhibited, from personal interview or medical records, significantly (** $p \leq 0.05$) greater chronic incidence of: diagnosed neurasthenia (80%**); self-reported dizziness (33%**); self-reported fatigue (43%**); diagnosed depression/lack of initiative (33%**); self-reported headache (17%); nausea (13%); palpitations/thoracic oppression (33%**); self-reported sleep disturbances (30%**); eye Irritancy (30%**); polyneuropathy (60%/NS); EEG alpha amplitude (**); simple reaction time across successive blocks (**); incidence of compulsive thoughts (**); and phobias (**).

Odkvist et al. (1987) examined the effects of chronic exposure to jet fuel on audiological and vestibulo-oculomotor function in 8 jet mechanics exposed from 15-41 years (mean = 25 years). All workers, aged 40-60, complained of neuropsychiatric symptoms consistent with those described previously for chronic exposure to jet fuels. Although there was no indication of any abnormal deficit in peripheral auditory mechanisms, on a battery of audiological function tests exposed workers exhibited significant deficit rates on Interrupted Speech Discrimination (38%**) and Cortical Response to Frequency Glides (50%**). The former test, sensitive to lesions of the central auditory pathways and auditory cortex, evaluated capacity of subjects to discriminate human speech interrupted at 4, 7 or 10 times per sec. The latter test, sensitive to lesions of the cerebello-pontine and other auditory pathways, required the subject to identify frequency glides in a 1000 Hz tone of 50 Hz or 200 Hz within 167 ms or 140 ms, respectively. The outcome of vestibulo-oculomotor tests indicated significant results on Broad-Frequency Visual Suppression (**) and Broad Frequency Smooth Pursuit (**) tests, but reflected no deficit in the sensory organs. For the former test, subjects were required to suppress the vesbulo-oculomotor reflex in order to fixate a dot on a full visual field screen moving with a rotating chair. Unlike during the testing of solvent exposed workers, jet fuel exposed workers did not exhibit a significant deficit on the Visual Suppression test until it was made more difficult by use of broad frequency pseudo-random swings. In the latter test, subjects were asked to follow a small target moving at a velocity of 10 or 25°/sec across a monitor, with the dependent measure being the eye speed between saccades. Values below 8 and 18°/sec, respectively, were considered significantly abnormal. This research appears to indicate the workers exposed chronically to jet fuel (mean = 25 years) may exhibit subtle deficits in the higher level inhibition (cerebellar, cortical, etc.) of brainstem functions, which may remain undetected without use of complex testing batteries. These deficits are consistent with, but of lesser severity than similar deficits commonly seen in some persons exposed chronically to industrial solvents (Odkvist et al., 1980, 1983; Hyden et al., 1983; Bergholtz et al., 1984; WHO, 1985).

Bogo et al. (1984) exposed Sprague-Dawley rats by whole body inhalation exposure for 6 hr/day, 5 days/wk, for 6 wk to aerosolized JP-5 (1100 mg/m^3), or by oral gavage to from 3-24 ml/kg JP-5 for one day, then evaluated the animals for neurobehavioral toxicity on the following tests: (a) somatosensory evoked potential (SEP) and cortical EEG; (b) food and water intake; (c) accelerod performance; (d) shock-elicited aggression; and (e) total locomotor activity. The authors reported, in the orally gavaged animals, reduced food and water intake for 2-7 days

post-exposure, significantly increased locomotor activity from 2-4 hr post-exposure, normal accelerod performance, and tactile hypersensitivity and self-mutilation, with normal shock-elicited aggression. The authors attributed the observed effects to possible gastrointestinal irritancy from the oral gavage exposure. The inhalation exposed rats exhibited a significant polydipsia that began 8 days into the exposure and persisted throughout the remaining 30 day experimental procedure, although no other neurobehavioral deficits were observed. Because no significant deficit in renal morphology or renal serum chemistry were observed in the polydipsic rats, the explanation for this significant behavioral change remains unclear.

The neural mechanisms underlying development of symptoms of neurotoxicity following jet fuel exposures are generally unknown and poorly researched. Bell et al. (1997a and 1997b) examined three models of development of neurotoxicity from exposure to potent olfactory stimuli: the olfactory-limbic and neural sensitization model; the kindling model; and the time-dependent sensitization model. In the first model, it is hypothesized that potent olfactory stimuli may sensitize the olfactory-limbic-mesolimbic brain regions either through sensory stimulation via the olfactory and trigeminal (irritancy) nerves, or through the infusion of chemicals from the nasal passages directly into the olfactory bulbs, where blood-brain barrier protection is minimal. In this model, the resultant CNS sensitization is manifested by increasingly more severe physiological or neurobehavioral consequences from subsequent exposure to the same, or different olfactory stimuli. In the kindling model, it is hypothesized that potent olfactory stimulation induces, over time, a partial (non-convulsive) kindling of the limbic system, such that subsequent olfactory stimulation may induce the electrophysiological (2-4 Hz EEG paroxysms) patterns of simple partial seizures. Finally, the non-kindling time-dependent sensitization (TDS) hypothesis contends that potent chemical stimuli (i.e., volatile organic compounds, stimulants, antidepressants, neuromodulators such as substance P, etc.) can induce a non-kindling increase in the responsivity of neural circuits, particularly as originate in the limbic system (Sorg et al., 1994; 1997). An explanation for amphetamine-induced sensitization of locomotor activity in animals is that the initial drug administrations induce TDS in circuits modulating the release of dopamine in areas influencing locomotor activity, such as the nucleus accumbens (NAc). Hence, in the TDS hypothesis repeated exposure to low levels of a chemical stimulus result in increasing observation of specific neurobehavioral responses without development of the EEG paroxysmal activity or motor seizures associated, respectively, with partial or full kindling processes. Additionally, there is limited evidence that jet fuel exposures may modulate expression of the detoxification liver enzyme glutathione S-transferase (Siegel, 1996), such that metabolism of toxicants may be generally reduced, and that JP-8 aerosol exposures in rodents may induce profound changes in the immune system (Harris et al., 1997a and 1997b).

METHODS AND MATERIALS (U)

ANIMALS (U)

(U) Groups of 32 male Sprague-Dawley^{CD} rats (250-300 g) obtained from Charles River Breeding Laboratories (Raleigh, NC) were exposed to JP-5 vapor, JP-8 vapor, or room air control (AC) conditions. Groups of 16 male Sprague-Dawley rats were exposed to JP-4 vapor or AC conditions. Subjects were individually housed in polycarbonate shoebox cages, and were allowed *ad lib* access to Purina rat chow and distilled water except during the 6-hr whole body inhalation exposures. Subjects were weighed on a daily basis through all experimental procedures and were examined by a member of the Veterinary Services staff.

FUELS (U)

(U) Jet propulsion (JP) fuels JP-4 (MIL-T-5624-L-Amd. 1) and JP-5 (MIL-T-5624M) samples were obtained from barrel stock at the Fuels Laboratory at WPAFB, OH and were verified by gas chromatographic (GC) analysis for purity. JP-8 samples were obtained from a test stock available at the WPAFB Fuels Laboratory, blended from JP-8 provided by several fuel manufacturers.

VAPOR GENERATION & EXPOSURES (U)

(U) JP-5, JP-8, and appropriate AC exposures occurred in three 270-L Hinners-type (Hinners et al., 1968) whole body inhalation chambers, in which groups of 16 rats each were exposed to either fuel vapor or room air for 6 hr/day (800-1400 hr), 5 days/wk (M-F), for six consecutive weeks (30 days, or 180 hr exposure). For the daily

exposures, rats were randomly placed in individual housing spaces within the two 8-rat wire mesh cages in each chamber, allowing maximal vapor distribution. For JP-4 vapor exposures, rats were similarly placed in one of four 8-rat mesh cages within one of two 670-L THRU whole body inhalation exposure chambers. For each exposure, raw fuel was directed through a heated J-tube, temperature controlled to maximize vaporization without formation of aerosol. The vaporized fuel was introduced into a (counter-current) room air source flowing into each 270-L chamber at 55-L per min, or into each 670-L chamber at 6 ft^3/min. Tubing directing the vapor flow was externally heated to minimize condensation, and contained a J-tube trap to contain and measure (approximately 2%) condensed vapor. Gelman 25-mm, extra thick, glass fiber filters were used to sample chamber atmospheres for aerosol. Chamber atmospheres were quantified by Infrared (IR) Spectrometry at 20 min intervals using the IR absorbence band between 3.4 and 3.5 microns calibrated with known mass concentrations of hexane. Chamber atmospheres were, then, adjusted by controlling raw fuel flow and/or J-tube temperature to maintain target concentrations $\pm$ 10%.

ANIMAL RESTING (U)

(U) Following completion of vapor or AC exposures, rats were rested in the home cages for 65 days (JP-8 and JP-5 exposures), or for 14 (50% of subjects) or 60 (50%) days following JP-4 exposures.

NEUROBEHAVIORAL TESTING (U)

(U) Following 14-65 days of resting, rats were evaluated for deficits in neurobehavioral capacity as measured by 9-10 tests selected from the NTAB (Ritchie et al., 1995). All rats experienced the battery of tests in the same order over approximately 21 days, but the order of experiencing individual tests was randomized within appropriate vapor exposure and AC groups. The following NTAB tests were utilized:

(1) Acoustic Startle Response (ASR): A well accepted measure of the integrity of auditory reflex centers in the mammalian brainstem, the ASR measures the amplitude of, time to, and habituation of startle responses to a series of 105 dB tones.

(2) Prepulse Inhibition of Acoustic Startle Response (PPI of ASR): A well accepted measure of inhibitory capacity of the mammalian brainstem, measuring the capacity of a low intensity (74 dB) warning tone occurring 300 ms prior to a higher intensity (105 dB) tone to reduce the "acoustic startle response" in rats to the louder tone. PPI of ASR is typically impaired in human schizophrenic patients (dopamine/serotonin imbalance).

(3) Appetitive Reinforcer Approach Sensitization (ARAS): A recently validated measure (Panksepp et al., 1997) of CNS sensitization, specifically of the dopaminergic system, in which the relative time spent by rats approaching a raw hamburger meat stimulus (after two previous opportunities to ingest hamburger) are compared to the approach to an unbaited stimulus.

(4) Forelimb Grip Strength (FGS): A measure of nigro-striatal system integrity, the FGS is the averaged (3 trials) forelimb grip strength as measured in rats by forced release (Kg force) of a mesh screen attached to a force dynamometer.

(5) Total Locomotor Activity (TLA): Using the Opto-Verimex apparatus (Columbus Instruments, Columbus, OH), nine measures of locomotor activity in rats (i.e., forward locomotion, rearing, stereotyped activity, resting, circling, etc.) are recorded for 3 successive 10-min periods.

(6) Light Sensitivity Test (LST): To evaluate acute or chronic light sensitivity (photophobia), rats are placed in a two chamber apparatus in which one chamber is illuminated (100W bulb) and one chamber is dark. Light sensitivity is measured by the relative percentages of time in which the subject resides in the light versus dark chamber.

(7) Tail Flick Response (TFR): To evaluate the nociceptive system of the rat, the animals are placed individually within a Plexiglas restraint tube then measured for the mean time required to exhibit a "tail flick" response following three temporally spaced dermal exposures of the proximal tail to the heat generated by a high intensity bulb located below a shutter.

(8) Conspecific Approach (CSA): The CSA test is a measurement of social cohesion in rats based on the time spent head-poking through a port allowing visual and olfactory access to a conspecific housed in an adjacent cage apparatus versus the time spent head-poking though a neutral port.

(9) Porsolt Forced Swim Test (PFST): A measure of depression or learned helpless in rats, the PFST is commonly used by the pharmaceutical industry to evaluate human anti-depressive drugs. In the PFST, rats are placed within a narrow cylinder filled with water to a level at which the rat must swim actively or float to avoid submersion. The length of time spent by the rat in active "escape" behaviors (swimming, diving, etc.) versus immobile floating during a 10 min test session is a measure of emotional depression. Additionally, by comparing the time to "immobility" on two successive days, the PFST provides a measurement of short-term memory.

(10) Passive Avoidance (PA): A commonly used test of memory in which rats are placed in the illuminated side of a two chamber apparatus, then allowed to enter the non-illuminated chamber. Upon entering the non-illuminated chamber, rats receive a punishing stimulus. Twenty-four hr following the initial training rats are placed back into the illuminated chamber, and the time to re-enter the non-illuminated chamber recorded as a measurement of memory consolidation.

(11) Morris Water Maze (MWM): A commonly used test of both spatial localization and memory in which rats are individually placed within a 48" diameter water tank in which the water has been made optically occlusive through the additional of non-toxic paint. The task of the rat is to locate and mount an "escape" platform which is submerged 3 cm below the water surface. Each subject is tested for 5 trials on Day 1, and the time to platform location and total distance traveled is recorded for each trial as a measurement of learning. On Day 2, each rat is returned to the same starting point within the apparatus. Time to platform location is recorded as an evaluation of spatial memory.

HISTOPATHOLOGY (U)

(U) Within 48 hr of completing the NTAB battery rats were sacrificed by decapitation, and serum and brain region samples (cerebellum, cortex, hippocampus, midbrain, brainstem) immediately collected and frozen. Tissues were analyzed for regional concentrations of: norepinephrine (NE), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin (5-hydroxytryptamine or 5-HT), homovanillac acid (HVA), and 5-hydroxyindole-acetic acid (5-HIAA) [methods described in Kim et al., 1987].

RESULTS AND DISCUSSION (U)

RESULTS (U)

Fig 1. Mean Rat Weights JP-5

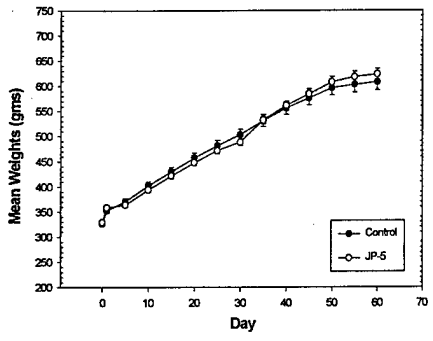
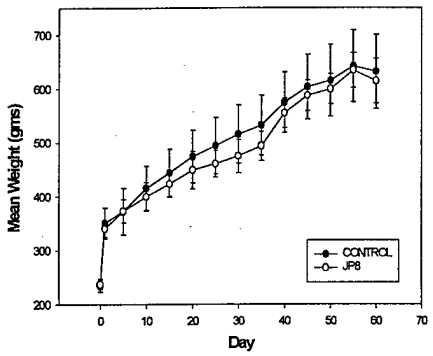


Fig. 2 Mean Rat Weights JP-8



(U) Rats Weights: Fig. 1 and 2 present mean rat weights ± SD for JP-5 and JP-8 exposures, respectively, as compared to weights for AC-exposed animals. During the period of exposure (Days 4-47), mean weights for both JP-5 and JP-8 exposed rats were numerically lower than for AC-exposed rats, although the differences were not statistically significant. Likewise, mean weights for JP-4 exposed rats were numerically lower than for AC-exposed rats during the period of exposure (data not shown).

(U) NTAB Tests: Table 1 summarizes the results of the NTAB evaluation of vapor-exposed and AC-exposed rats for testing of JP-8, JP-5 and JP-4. Entries marked with ** indicate significant differences ($p < 0.05$)between the means for fuel vapor and AC exposures. NE indicates no significant difference between the means for fuel-exposed and AC rats. NT indicates that rats in the indicated exposure condition were not evaluated with the NTAB test referenced.

NTAB Test	JP-8 65 Day	JP-5 65 Day	JP-4 14 Day	JP-4 60 Day
Acoustic Startle Response (ASR)	NE	NE	NE	NE
Prepulse Inhibition (PPI of ASR)	NE	NE	NE	**
Appetitive Stimulus Approach Sensitization (ARAS)	**	NE	**	NE
Forelimb Grip Strength (FGS)	**	NE	NE	NE
Total Locomotor Activity (TLA)	NE	NE	NE	**
Light Sensitivity Test (LST)	NT	NT	NE	NE
Tail Flick Response (TFR)	NE	NE	NE	**
Conspecific Approach (CA)	NE	NE	NT	NT
Porsolt Forced Swim Test (PFST)	NE	NE	NT	NT
Passive Avoidance (PA)	NE	NE	NE	NE
Morris Water Maze (MWM)	NE	NE	NT	NT

(U) Results of Individual NTAB Tests: The following figures display results wherein significant differences ($p < 0.05$) on individual NTAB tests were detected between fuel vapor-exposed and AC groups:

Fig. 3. Total Locomotor Activity (JP-4 Vapor Exposures)

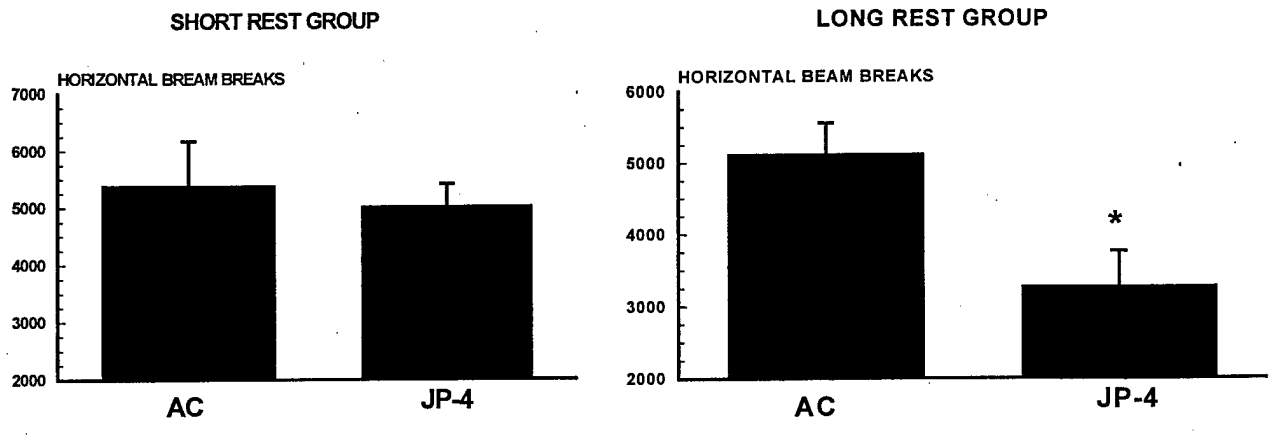


Fig. 4. Tail Flick Response (JP-4 Exposures)

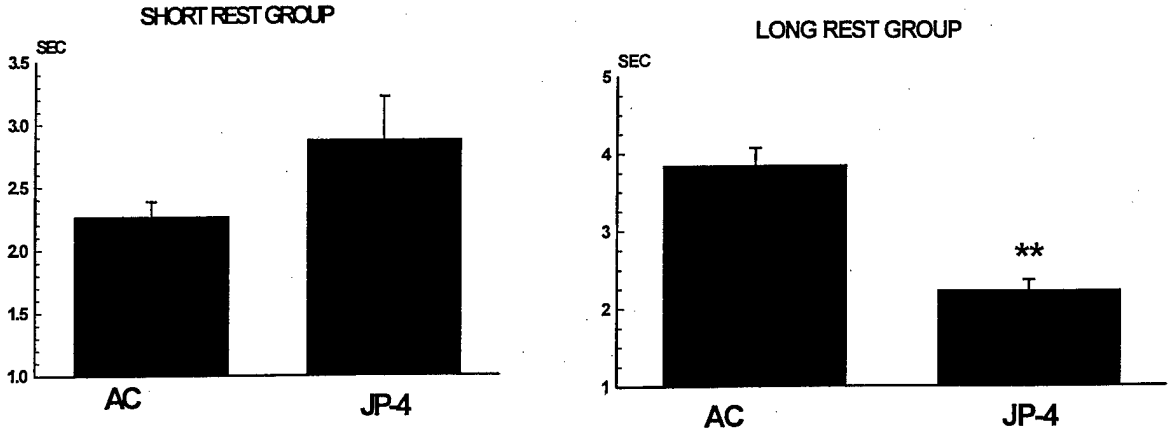


Fig. 5. Prepulse Inhibition of the Acoustic Startle Response (JP-4 Exposures)

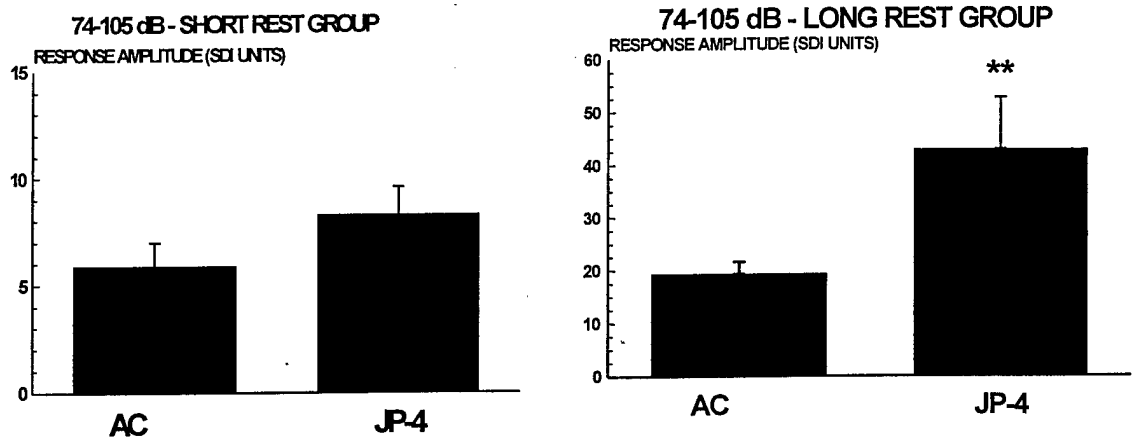
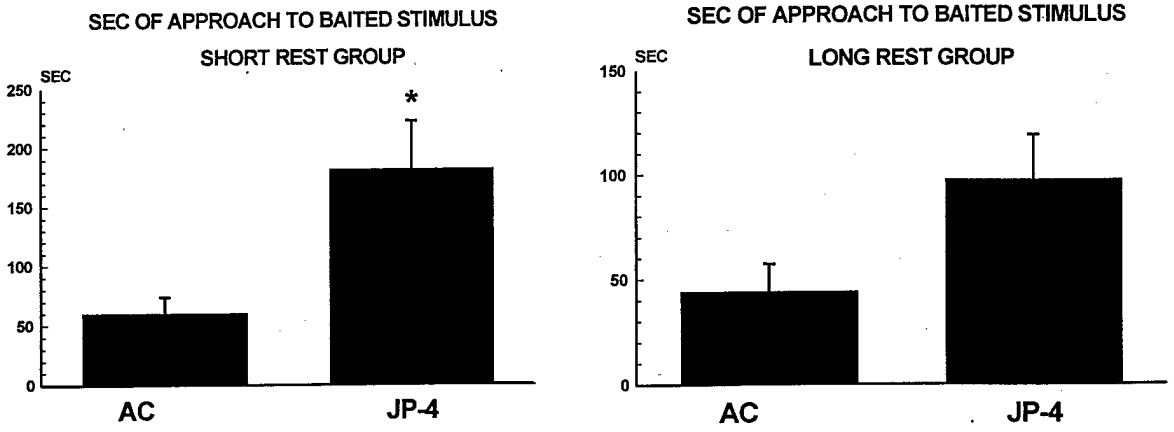
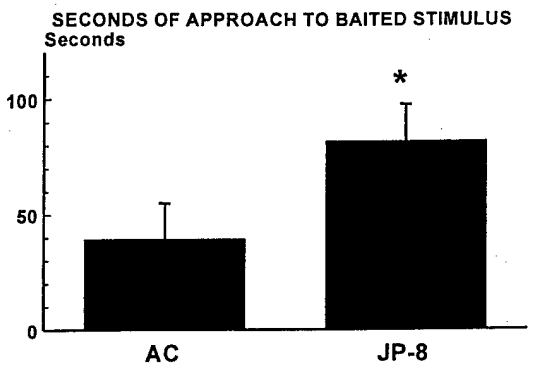


Fig. 6. Appetitive Reinforcer Approach Sensitization (JP-4 and JP-8 Exposures)





Regional CNS Neurotransmitter and Metabolite Concentrations (U): Table 2 indicates significant differences between means for neurotransmitter and major metabolite concentrations in measured regions for fuel vapor-exposed and AC rats. The direction of the arrow indicates whether the difference in neurotransmitter or metabolite concentrations was increased (⇑) or decreased (⇓) relative to the mean for the appropriate AC comparison.

Table 2. Significant differences ($p < 0.05$) in neurotransmitter or metabolite levels in brain regions (ng neurotransmitter/mg protein ± SD)

Neurotrans-mitter or Metabolite	JP-4 Vapor 60 Day	Control (JP-4) 60 Day	JP-5 Vapor 65 day	Control (JP-5) 65 day	JP-8 Vapor 65 Day	Control (JP-8) 65 Day
Dopamine (DA)			Cortex 0.604±0.067 ⇑	Cortex 0.410±0.052	Cerebellum 0.419±0.045 ⇓ Brain Stem 0.226±0.015 ⇓	Cerebellum 0.657±0.195 Brain Stem 0.284±0.046
DOPAC			Hippocamp 1.908±0.767 ⇑	Hippocamp 1.280±0.422	Midbrain 1.237±0.363 ⇑	Midbrain 0.800±0.292
Serotonin (5-HT)	Cerebellum 0.93±0.04 ⇑ Brainstem 0.90±0.01 ⇑ Hippocamp 0.94±0.08 ⇑ Cortex 0.92±0.07 ⇑	Cerebellum 0.83±0.03 Brainstem 0.75±0.05 Hippocamp 0.78±0.04 Cortex 0.81±0.04				
HVA	Not Measured	Not Measured	Hippocamp 0.242±0.096 ⇑	Hippocamp 0.162±0.045		
5-HIAA	Cerebellum 3.61±0.12 ⇑ Brainstem 2.27±0.22 ⇑ Striatum 3.77±0.12 ⇑ Cortex 2.24±0.15 ⇑	Cerebellum 2.79±0.23 Brainstem 1.78±0.12 Striatum 3.07±0.38 Cortex 1.69±0.28				

DISCUSSION (U)

(U) Previous (JP-4 or JP-5), ongoing (JP-5 or JP-8), or future exposure of personnel to jet fuel during careers lasting from 3-20+ years is an issue of concern to the operational military. Personnel may be exposed acutely (e.g., accidental spillage), subchronically (e.g., short-term combat deployment), or on a daily basis (occupational) to varying concentrations of one or more jet fuels. These exposures are typically by respiratory inhalation, to vapor and/or aerosol, but may include dermal absorption and oral ingestion. These exposures may occur to only jet fuel, but more commonly may occur during co-exposure to other potential toxicants (lubricants, solvents, insecticides, etc.) and stressors (i.e., physical fatigue, heat or cold stress).

In the studies discussed, adult rats were exposed by whole body inhalation to JP-4, JP-5 or JP-8 jet fuel vapor (1.0 - 2.0 mg/L) for from 84-180 hr, over 14-30 days. Rats were exposed to vapor concentrations that induced no observable irritancy response or neurobehavioral deficit, and were selected to simulate possible real world exposure of military personnel during workplace or combat deployment. During all vapor exposures, the mean weights of the exposed groups were numerically lower than appropriate control groups, indicating a slightly reduced growth rate, although recovery to control levels occurred rapidly following completion of exposures. Following rest periods of from 14-65 days, rats were evaluated on a battery of neurobehavioral tests developed to identify performance deficits that may be topographically similar to those reported in humans exposed chronically to varying concentrations of jet fuel. The rest periods were utilized to minimize neurobehavioral effects that may reflect physiological irritancy or stress from the exposure procedures. Additionally, the rest periods were employed to ensure that neurobehavioral deficits that may not be exhibited until several weeks post-exposure were not ignored. Human syndromes that may be related to chemical exposures (Multiple Chemical Sensitivity, for example) often do not express major symptomology until weeks or months following the initial exposure. Finally, regional brain tissues were examined for changes in the concentration of neurotransmitters and major metabolites in an attempt to identify biomarkers of any performance changes detected.

In the first study discussed (published as Nordholm et al., 1999), it was shown that whole body inhalation exposure of Sprague-Dawley rats to JP-4 vapor (2.0 mg/L) for 6 hr/day for 14 consecutive days resulted in a significant decrease in prepulse inhibition of the acoustic startle response, a significant decrease in the mean time to tail flick nociceptive response, a significant decrease in spontaneous locomotor activity, and a significantly increased approach to a "novel" appetitive stimulus. Each of these changes in performance capacity have been previously associated with changes in levels of CNS dopamine and/or serotonin (Nordholm et al., 1999). Reduced prepulse inhibition in humans is, for example, a major neurobehavioral marker of schizophrenia, and is typically associated with dopaminergic or serotonergic imbalance. As was shown, the performance changes in rats exposed to JP-4 vapor were associated with significantly increased levels of serotonin in the cerebellum, brainstem, hippocampus and cortex, and increased levels of the major serotonin metabolite 5-HIAA in the cerebellum, brainstem, striatum and cortex. While dopamine and DOPAC levels in these rats, sacrificed approximately 36 (14 day rest group) or 82 (60 day rest group) days post-exposure, were not significantly different from controls it is not known whether dopamine levels may have been elevated or reduced during the periods of resting and neurobehavioral testing in these animals. There are a number of published studies measuring the induction of dopamine release by changes in serotonin concentrations (Bonhomme et al., 1995; De Deurwaerdere et al., 1996), highlighting possible interactive changes in CNS neurotransmitter balance as a function of chemical exposures that may directly influence only one neurotransmitter system.

In the present studies, it was shown that whole body inhalation exposure of Sprague-Dawley rats to JP-8 (1.0 mg/kg) or JP-5 (1.2 mg/kg) vapor resulted in changes in neurobehavioral capacity and/or levels of CNS neurotransmitters. JP-8 exposures resulted in a significant increase in approach to a "novel" appetitive stimulus in the ARAS test and a significant increase in the forelimb grip strength (FGS), consistent with significant decreases in the level of dopamine measured at approximately 88 days post-exposure in both the cerebellum and brainstem, and a significant increase in DOPAC in the midbrain region, as compared to air controls. JP-5 exposures resulted in no significant deficits on any of the NTAB tests, but nevertheless significantly increased levels of dopamine and its major metabolite DOPAC in the cortex and hippocampus, respectively, and significantly increased levels of the serotonin metabolite homovanillac acid (HVA) in the hippocampus.

It cannot, of course, be assumed that there exists a correlative relationship between the reported changes in neurobehavioral capacity and in neurotransmitter/metabolite levels. There is, however, a substantial scientific literature relating acute or repeated exposure to the known neurotoxic components of jet fuels (toluene, benzene, xylene, trimethylbenzene, n-hexane, etc.) [Benignus, 1981; WHO, 1985] and alteration in performance capacity, as well a significant literature relating neurobehavioral deficits and modulation of CNS neurotransmitter levels. Exposure of animals or humans to toluene has, for example, been implicated in motor performance syndomes (Pryor, 1991; von Euler et al., 1993; Rahill et al., 1996); audiological deficits (Bergholtz and Odkvist, 1984), including detection of auditory signals (Bushnell et al., 1994); visuo-vestibular and oculomotor deficits (Odkvist et al., 1980; Odkvist et al., 1983; Hayden et al., 1983; Niklasson et al., 1995); deficits in schedule-controlled behavior, as well as in working and reference memory (Ikeda et al., 1993; Miyagawa et al., 1995), including spatial learning and memory (von Euler et al., 1993); and induction of human psychoorganic syndrome (Forkman et al., 1991). Changes in neurotransmitter levels and neurotransmitter/metabolite ratios, emphasizing serotonin and dopamine, have been correlated with numerous changes in neurobehavioral capacity in animals and humans, including nociceptive transmission (Alhaider and Wilcox, 1993; Bervoets et al., 1993); arousal and activity levels (Chojnacka-Wojcik, 1992; Geyer, 1996); and sensorimotor gating of the startle response (Geyer and Braff, 1987; Rigdon and Witherspoon, 1992; Sipes and Geyer, 1995).

It appears, then, that exposure of adult Sprague-Dawley rats to JP-4, JP-5 or JP-8 vapor (without aerosol) for at from 14-30 days may be adequate to induce persisting changes in neurobehavioral capacity and/or changes in dopaminergic and/or serotonergic systems in the brain. It is important to note that observational evaluation of the exposed rats in all groups detected no vapor-induced changes, except a numerical reduction of weight gain throughout the periods of exposure that were rapidly reversed post-exposure. Use of a number of NTAB tests, however, identified several subtle changes in neurobehavioral capacity that appear related to the specific vapor exposure and, at least in the case of JP-4, to the time post-exposure that the tests were conducted. JP-4 vapor induced changes in approach to a "novel" stimulus (ARAS) were, for example, observed in animals tested after a 14 day rest, but not following a 60 day rest, while changes in prepulse inhibition of the acoustic startle response, tail flick nociception, and locomotor activity were measured only in the rats rested for 60 days. The CNS mechanisms underlying the neurobehavioral changes observed in rats following 14 day versus 60 day rest periods may be explained by changes in neurotransmitter levels or activity that varied as a function of post-exposure time. (Nordholm et al., 1999).

Comparing results from NTAB evaluation of rats exposed to JP-4, JP-5 or JP-8 vapor, it would appear that JP-4, a jet fuel formulation that is no longer utilized by the US or other NATO nations, exhibits more potential for neurobehavioral toxicity than does JP-5 or JP-8. While JP-5 jet fuel remains widely used by the US Navy/Marine Corps, there is an ongoing effort to replace all JP-5 applications with use of JP-8. While JP-8 was shown to induce more significant changes in performance capacity than did JP-5 on the selected NTAB tests, it cannot be stated unequivocally that there exists a significant difference in the potential for induction of neurobehavioral toxicity in rats. It should be remembered that exposure of rats to JP-5 or JP-8 vapor induced unique alterations in the regional concentrations of serotonin and/or dopamine. While no neurobehavioral deficits were measured following repeated JP-5 exposures, this may simply reflect the selection of tests from the NTAB battery.

It cannot, of course, be assumed that jet fuel vapor-induced changes in the neurobehavioral capacity and neuromolecular activity of Sprague-Dawley rats should be expected in fuel vapor-exposed humans. The neurobehavioral deficits measured in the fuel vapor-exposed rats, however, bear some topographical similarity to the neurobehavioral and psychological deficits reported in European jet engine manufacturing personnel and in others exposed to jet fuels repeatedly for many years, and justify continuing research efforts.

SUMMARY AND CONCLUSIONS (U)

1. Repeated exposure (14 to 30 days) of rats to JP-4, JP-5, or JP-8 vapor resulted in numerically reduced weight gains, compared to animals exposed to room air conditions, that persisted during and for several days following the exposures.

2. Repeated exposure to JP-4, JP-5, or JP-8 vapor resulted in no visually observable signs of physiological irritancy (ocular, dermal, respiratory) nor any observable changes in neurobehavioral capacity (e.g., motor signs, ease of handling, responsiveness to stimuli, etc.).

3. Exposure to JP-4 (84 hr at 2.0 mg/L) vapor resulted in persisting deficits on the PPI of ASR, ARAS, TLA, and the TFR tests that varied as a function of time post-exposure, and were consistent with significant elevations in 5-HT and 5-HIAA compared to AC controls.

4. Exposure to JP-8 (180 hr at 1.0 mg/L) vapor resulted in deficits on the ARAS and FGS tests, consistent with significant changes in dopamine or DOPAC levels in several brain regions.

5. Although exposure to JP-5 (180 hr at 1.2 mg/L) resulted in no significant effects on NTAB tests, significant elevations in dopamine and DOPAC, and in the serotonin metabolite HVA where measured in several brain regions.

6. Based on NTAB analysis of fuel vapor-induced changes in rodents, it would appear that the comparative neurobehavioral risk from exposures is JP-4 > JP-8 $\geq$ JP-5, although it cannot, of course, be assumed that the selected NTAB tests measured all performance deficits resulting from the exposures.

REFERENCES (U)

Alhaider, A.A., and Wilcox, G.L. 1993. Differential roles of 5-hydroxytryptamine1A and 5- hydroxytryptamine1B receptor subtypes in modulating spinal nociceptive transmission in mice. *J. Pharmacol. Exp. Ther.* 265:378-385.

ATSDR. 1993. *Draft Toxicological Profile for Jet Fuels JP-4 and JP-7.* Atlanta, GA: Agency for Toxic Substances and Disease Registry, Centers for Disease Control.

Bakshi, K. S., and Henderson, R. F. 1998. Permissible exposure levels for selected military fuel vapors. *Inhalation Toxicol.* 10:955-961.

Bell, I.R., Schwartz, G.E., Baldwin, C.M., Hardin, E.E., Klimas, N.G., Kline, J.P., Patarca, R., and Song, B. 1997a. Individual differences in neural sensitization and the role of context in illness from low-level environmental chemical exposures. *Environ. Health Perspect. Supple.* 105:457-456.

Bell, I.R., Rossi III, J., Gilbert, M.E., Kobal, G., Morrow, L.A., Newlin, D.B., Sorg, B.A., and Wood, R. 1997b. Testing the neural sensitization and kindling hypothesis for illness from low levels of environmental chemicals. *Environ. Health Perspect.* 105 Suppl. 2:539-547.

Benignus, V. A. 1981. Health effects of toluene: a review. *Neurotoxicol.* 2:567-588.

Bergholtz, L. M., and Odkvist, L. M. 1984. Audiological findings in solvent exposed workers. *Acta Otolaryngologic, Supplement* 412:109-110.

Bergin, P.S., Bronstein, A.M., Murray, N.M., Sancovic, S., and Zeppenfeld. 1995. Body sway and vibration perception thresholds in normal aging and in patients with polyneuropathy. *J. Neurol. Neurosurg. Psychiatry* 58:335-340.

Bervoets, K., Rivet, J. M., and Millan, M. J. 1993. 5-HT1A receptors and the tail-flick response. IV. Spinally localized 5-HT1A receptors postsynaptic to serotoninergic neurons mediate spontaneous tail-flicks in the rat. *J. Pharmacol. Exp. Ther.* 264:95-104.

Bogo, V., Young, R.W., Hill, T.A., Cartledge, R.M., Nold, J. and Parker, G.A. 1984. Neurobehavioral toxicology of petroleum- and shale-derived jet propulsion fuel No. 5 (JP5). In M.A. Mehlman (ed.), *Applied Toxicology of Petroleum Hydrocarbons, Vol. VI.* Princeton Scientific Publishers, Princeton, NJ. pp. 17-33.

Bonhomme, N., De Deurwaerdere, P., Le Moal, M., and Spampinato, U. 1995. Evidence for 5-HT4 receptor subtype involvement in the enhancement of striatal dopamine release induced by serotonin: a microdialysis study in the halothane-anesthetized rat. *Neuropharmacol.* 34:269-279.

Burbacher, T. M. 1993. Neurotoxic effects of gasoline and gasoline constituents. *Environ. Health Perspect.* 101 Suppl 6:133-141.

Bushnell, P. J., Kelly, K. L., and Crofton, K. M. 1994. Effects of toluene inhalation on detection of auditory signals in rats. *Neurotoxicol. Teratol.* 16:149-160.

Clark, C.R., Ferguson, P. W., Katchen, M. A., et al. 1989.Comparative acute toxicity of shale and petroleum derived distillates. *Toxicol. Ind. Health* 5:1005-1016.

Chojnacka-Wojcik, E. 1992. Involvement of dopamine autoreceptors in the hypoactivity induced by 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) in mice. *Pol. J. Pharmacol. Pharm.* 44:135-146.

Clinger, O.W., and Johnson, N.H. 1951. Purposeful inhalation of gasoline vapors. *Psychiatr. Q.* 25:557-567.
COT (Committee on Toxicology), National Research Council. 1996. *Permissible Exposure Levels for Selected Military Fuel Vapors.* Washington, DC.: National Academy Press.

Davies, N. E. 1964. Jet fuel intoxication. *Aerospace Med.* 35:481-482.

De Deurwaerdere, P., Bonhomme, N., Lucas, G., Le Moal, M., and Spampinato, U. 1996. Serotonin enhances striatal dopamine outflow in vivo through dopamine uptake sites. *J. Neurochem.* 66:210-215.

Easson, W. M. 1962. Gasoline addiction in children. *Pediatrics* 29:250-254.

Flodin, U., Edling, C., and Axelson, O. 1984. Clinical studies of psychoorganic syndromes among workers with exposure to solvents. *Am. J. Ind. Med.* 5:287-295.

Forkman, B. A., Ljungberg, T., Johnson, A. C., Nylen, P., Stahle, L., Hoglund, G., and Ungerstedt, U. 1991. Long-term effects of toluene inhalation on rat behavior. *Neurotoxicol. Teratol.* 13:475-481.

Gaworski, C.L., MacEwen, J.D., Vernot, E.H., Bruner, R.H., and Cowan, M.J. 1984. Comparison of the subchronic inhalation toxicity of petroleum and oil shale JP-5 jet fuels. In: M.A. Mehlman, ed. *Applied Toxicology of Petroleum Hydrocarbons* Vol. 6, pp. 33-47. New Jersey: Princeton Scientific Publishers, Inc.

Geyer, M. A. 1996. Serotonergic functions in arousal and motor activity. *Behav. Brain Res.* 73:31-35.

Geyer, M. A., and Braff, D. L. 1987. Startle habituation and sensorimotor gating in schizophrenia and related animal models. *Schizophr. Bull.* 13:643-668.

Grasso, P. 1988. Neurotoxic and neurobehavioral effects of organic solvents on the nervous system. *Occupational Medicine: State of the Art Reviews* 3:525-539.

Harris, D.T., Sakiestewa, D., Robledo, R. F., and Witten, M. 1997a. Protection from JP-8 jet fuel induced immunotoxicity by administration of aerosolized substance P. *Toxicol. Ind. Health* 13:571-588.

Harris, D.T., Sakiestewa, D., Robledo, R. F., and Witten, M. 1997b. Short-term exposure to JP-8 jet fuel results in long-term immunotoxicity. *Toxicol. Ind. Health* 13, ISS 5:559-570.

Hayden, D., Larsby, B., Andersson, H., Odkvist, L.M., Liedgren, S.R.C., and Tham, R. 1983. Impairment of the visuo-vestibular interaction in humans exposed to toluene. *O.R.L.* 45:262-269.

Hinners R.G., Burkhart J.Y., and Punte E.C. 1968. Animal inhalation exposure chambers. *Arch Environ Health* 16:194-206.

Holm, S., Norback, D., Frenning, B., and Gothe, C. J. 1987. Hydrocarbon exposure from handling jet fuel at some Swedish aircraft units. *Scand. J. Work Environ. Health* 13:438-444.

Ikeda, T., Katakura, Y., Kishi, R., and Miyake, H. T. 1993. Acute neurobehavioral effects of co-inhalation of toluene and n-hexane on schedule-controlled behavior in rats. *Environ. Res.* 63:70-81.

Kearney, C.A., and Dunham, D.B. 1986. Gasoline vapor exposures at a high volume service station. Am. Ind. Hyg. Assoc. 47:535-539.

Kim, C., Speisky, M. B., Kharouba, S. N. 1987. Rapid and sensitive method for measuring norepinephrine, dopamine, 5-hydroxytryptamine and their major metabolites in rat brain by high-performance liquid chromatography. Differential effect of probenecid, haloperidol and yohimbine on the concentrations of biogenic amines and metabolites in various regions of rat brain. *J. Chromatogr.* 386:25-35.

Kinkead, E. R., DiPasquale, L. C., Vernot, E. H., et al. 1974. Chronic toxicity of JP-4 jet fuel. *Proceedings of the Annual Conference on Environmental Toxicology.* 5:145-154.

Knave, B., Persson, H. E., Goldberg, J. M., and Westerholm, P. 1976. Long-term exposure to jet fuel: an investigation on occupationally exposed workers with special reference to the nervous system. *Scand. J. Work Environ. Health* 2:152-164.

Knave, B., Olson, B. A., Elofsson, S., Gamberale, F., Isaksson, A., Mindus, P., Persson, H. E., Struwe, G., Wennberg, A., and Westerholm, P. 1978. Long-term exposure to jet fuel. II. A cross-sectional epidemiologic investigation on occupationally exposed industrial workers with special reference to the nervous system. *Scand. J. Work Environ. Health 4*:19-45.

Knave, B., Mindus, P., and Struwe, G. 1979. Neurasthenic symptoms in workers occupationally exposed to jet fuel. *Acta Psychiatr. Scand.* 60:39-49.

Macys, D. A., Carpenter, R. L., Risher, J. F., Vinegar, A., Dodd, D. E., and Wall, H. G. 1992. Results of a workshop on health effects of crude oil exposures related to Operation Desert Storm. *Naval Medical Research Institute, Government Publication*, NMRI 92-04.

Maruff, P., Burns, C.B., Tyler, P., Currie, B.J., and Currie. J 1998. Neurological and cognitive abnormalities associated with chronic petrol sniffing. *Brain.* 121:1903-1917.

Miyagawa, M., Honma, T., and Sato, M. 1995. Effects of subchronic exposure to toluene on working and reference memory in rats. *Neurotoxicol. Teratol.* 17:657-664.

Niklasson, M., Stengard, K., and Tham, R. 1995. Are the effects of toluene on the vestibulo- and opto-ocular motor system inhibited by the action of $GABA_B$ antagonist CGP 35348. *Neurotoxicol. Teratol.* 17:351-371.

Nordholm, A.F., Rossi III, J., et al. 1999. Repeated exposure of rats to JP-4 vapor induces changes in neurobehavioral capacity and 5HT/5-HIAA levels. *J. Toxicol. Environ. Health* 56:471-499.

Nuttall, J.B. 1958. Toxic hazards in the aviation environment. *J. Aviat. Med.* 29:641-649.

Odkvist, L.M., Larsby, B., Fredrickson, J.M., Liedgren, S.R.C., and Strand, E. 1980. Vestibular and oculomotor disturbances caused by industrial solvents. *J. Otolaryngol.* 9:53-59.

Odkvist, L.M., Larsby, B., Tham, R., and Hayden, D. 1983. Vestibulo-oculomotor disturbances caused by

industrial solvents. *Otolaryngol. Head Neck Surg.* 159:326-338.

Odkvist, L. M., Arlinger, S. D., Edling, C., Larsby, B., and Bergholtz, L. M. 1987. Audiological and vestibulo-oculomotor findings in workers exposed to solvents and jet fuel. *Scand. Audiol.* 16:75-81.

Page N.P., and Mehlman, M. 1989. Health effects of gasoline refueling vapors and measured exposures at service stations. *Toxicol. Indust. Health* 5:869-889.

Panksepp, J., Nelson, E., and Bekkedal, M. 1997. Brain systems for the mediation of social separation-distress and social-reward. Evolutionary antecedents and neuropeptide intermediaries. *Ann. N.Y. Acad. Sci.* 807:78-100.

Porter, H.O. 1990. Aviators intoxicated by inhalation of JP-5 fuel vapor. *Aviat. Space Environ. Med.* 61:654-656.

Pryor, G. T. 1991. A toluene-induced motor syndrome in rats resembling that seen in some human solvent abusers. *Neurotoxicol. Teratol.* 13:387-440.

Rahill, A. A., Weiss, B., Morrow, P. E., Frampton, M. W., Cox, C., Gibb, R., Gelein, R., Speers, D., and Utell, M. J. 1996. Human performance during exposure to toluene. *Aviat. Space Environ. Med.* 67:640-671.

Rigdon, C. C., and Witherspoon, J. K. 1992. 5-Hydroxytryptamine 1a receptor agonists block prepulse inhibition of acoustic startle reflex. *J. Pharmacol. Exp. Ther.* 263:486-493.

Ritchie, G. D., Rossi III, J., Macys, D. A., and Still, K. R. 1995. Application of the NMRI/TD Neurobehavioral Screening Battery to combustion toxicology. In *Fire and Polymers II: Materials and tests for Hazard Prevention*, ed. G. Nelson, pp. 344-366. Washington, D.C.: ACS Books.

Selden, A., and Ahlborg, G. 1991. Mortality and cancer morbidity after exposure to military aircraft fuel. *Aviat. Space Environ. Med.* 62:789-794.

Shalka, D. 1989. *Exposure to JP-8 Fuel Vapor and Its Combustion Products.* Brooks AFB, TX: USAF OEH/ECH.

Sipes, T. A., and Geyer, M. A. 1995. 8-OH-DPAT disruption of prepulse inhibition in rats: reversal with (+)WAY 100,135 and localization of site of action *Psychopharmacology Berl.* 117:41-81.

Smith, L. B., Bhattacharya, A., Lemasters, G., Succop, P., Puhala, E. 2nd, Medvedovic, M., and Joyce J. 1997. Effect of chronic low-level exposure to jet fuel on postural balance of US Air Force personnel. *J. Occup. Environ. Med.* 39:623-632.

Sorg, B. A., Hooks, M. S., Kalivas, P. W. 1994. Neuroanatomy and neurochemical mechanisms of time-dependent sensitization. *Toxicol. Ind. Health* 10:369-386.

Sorg, B. A., Willis, J. R., Nowatka, T. C., Ulibarri, C., See, R. E., and Westberg, H. H. 1996. Proposed animal neurosensitization model for multiple chemical sensitivity in studies with formalin. *Toxic.* 111:135-145.

Spencer, P.S. 1984. Experimental evaluation of selected petrochemicals for subchronic neurotoxic properties. In M.A. Mehlman (ed.), *Applied Toxicology of Petroleum Hydrocarbons, Vol. VI.* Princeton Scientific Publishers, Princeton, NJ. pp. 17-33.

Struwe, G., Knave, B., and Mindus, P. 1983a. Neuropsychiatric symptoms in workers occupationally exposed to jet fuel-a combined epidemiological and casuistic study. *Acta Psychiatr. Sçand. Suppl.* 303:55-67.

Struwe, G., and Wennberg, A. 1983b. Psychiatric and neurological symptoms in workers occupationally exposed to organic solvents - results of a differential epidemiological study. *Acta Psychiatr. Scand.* 67 (Suppl. 303):68-80.

Vernot, E. H., Drew, R. T., and Kane, M. L. 1990. Acute toxicological evaluation of jet fuel A. *Acute Toxicology*

Data 12:29-30.

von Euler, G., Ogren, S. O., Li, X. M., Fuxe, K., and Gustafsson, J. A. 1993. Persistent effects of subchronic toluene exposure on spatial learning and memory, dopamine-mediated locomotor activity and dopamine D2 agonist binding in the rat. *Toxicology* 77: 223-232.

WHO. 1985. *Chronic Effects of Organic Solvents on the Central Nervous System and Diagnostic Criteria.* WHO Regional Office, Copenhagen, and the Nordic Council of Ministers, Oslo.

Wolfe, R. E., Kinead, E. R., Feldmann, M. L., Leahy, H. F., and Jederberg, W. W. 1997. Acute toxicity evaluation of JP-8 jet fuel and JP-8 jet fuel containing additives. *Govt. Reports Announcements & Index (GRA&I),* Issue 09.